MECHANISMS OF DECREASED PLASMA VOLUME DURING ACUTE PSYCHOLOGICAL STRESS AND POSTURAL CHANGE IN HUMANS

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Abstract

Title of Dissertation: Mechanisms of Decreased Plasma Volume During Acute Psychological Stress and Postural Change in Humans

Stephen Michael Patterson, Doctor of Philosophy, 1993

Dissertation directed by: David S. Krantz, Ph.D., Professor, Department of Medical Psychology

Recent research has noted that acute psychological stress can lead to rapid and significant decreases in plasma volume in humans. However, this research has not conclusively identified the underlying mechanisms for stress-induced plasma volume changes. The present research examined the effects of psychological stress on plasma volume and the relationship of these changes to increases in blood pressure, red blood cell mass, and red blood cell fluid volume. The time course of plasma volume changes following psychological stress was also assessed. Plasma volume change was assessed using mass densitometry techniques, thus allowing for direct assessment of plasma volume.

In this study, 10 men and 10 women were evaluated for their hematologic and hemodynamic responses in response to a stressful math task and to postural change (standing). A no-stress control group of 10 men and 10 women performed a non-stressful reading task and the standing task. The two tasks (mental arithmetic or reading, and standing) were counterbalanced within each group. Results indicated that

psychological stress and posture change produced significant decreases in plasma volume and significant increases in blood pressure, blood and plasma density, and total plasma protein. No gender differences were found for lean body weight-corrected changes in plasma volume, blood pressure, or any other hematologic factor during any of the tasks. A reliable association was observed between blood pressure responses and plasma volume changes during psychological stress, and plasma volume returned to baseline within 12 minutes following mental arithmetic.

The present results suggest that an important mechanism for psychological stress-induced decreases in plasma volume is increased blood pressure leading to increased transvascular fluid shifts from the vascular system into the interstitial spaces. Support for this mechanism also comes from the rapid return of plasma volume to baseline levels following psychological stress, which corresponds with the rapid return of blood pressure once the stressor was terminated. The potential importance of stress-mediated decreases in plasma volume for psychophysiological research is discussed.

MECHANISMS OF DECREASED PLASMA VOLUME DURING ACUTE PSYCHOLOGICAL STRESS AND POSTURAL CHANGE IN HUMANS

by

Stephen Michael Patterson

Dissertation submitted to the
Faculty of the Department of Medical Psychology
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DEDICATION

To my wife and children - Maria, Christina, Fernando, Camilla, and Julian - for all the support, encouragement, and love throughout one of the most difficult and trying periods of our lives.

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INTRODUCTION

The experimental study of the effects of psychological stress on constituents of blood involved in hemostasis (the arrest of vascular bleeding) and thrombosis (blood clot development) began with the early work of Walter Cannon. In his research on the "fight or flight" response, Cannon (1929) noted that, among other physiological changes (e.g., increased heart rate and respiration), the number of red blood cells in circulation increased. Cannon felt that this was an appropriate component of the "fight or flight" response in that it increased the oxygen supply to the musculature of the arms and legs for added strength and endurance. Similarly, Selye (1956) observed that one of the components of the "non-specific" pattern of physiological reaction to various noxious stimuli was an increase in the number of white blood cells in circulation, which aided in protecting the organism during the alarm and resistance stage of the general adaptation syndrome.

Over the past 30 years, continued research on the physiological effects of stress and its role in the pathogenesis of different disease states has indicated that psychological stress affects the concentration of several different constituents of the blood. Several studies have found that acute psychological stress can produce increases in platelets (Jern, Wadenvik, Mark, Hallgren, & Jern, 1988; Jern, Jern, & Wadenvik, 1991), fibrinogen (Jern et al, 1989), plasma

proteins (Patterson, Krantz, Gottdiener, Hecht, Vargot, & Goldstein, under review), and serum cholesterol concentrations in the blood (Dimsdale & Herd, 1982; Niaura, Stoney, & Herbert, 1992).

Another relevant blood factor is plasma volume, and decreases in plasma volume and increased hemoconcentration during postural change as well as the hydrostatic pressure mechanism for posture-induced decreases in plasma volume have been recognized for years (Thompson, Thompson, & Dailey, 1929; Waterfield, 1931). The cause of these changes is an increase in vascular pressure due to increased gravitational forces exerted on the lower extremities after an individual changes from a seated to standing position. More recently, it has been observed that psychological stress can also cause an acute decrease in the volume of plasma found in the blood and a subsequent hemoconcentration of blood cells (Jern et al, 1991), cholesterol, and plasma proteins in healthy men (Muldoon, Bachen, Manuck, Waldstein, Bricker, & Bennett, 1992; Patterson, Gottdiener, Hecht, Vargot, & Krantz, in press). However, inconsistencies have been observed in the time course of this stress-induced plasma volume change. Both Jern et al. (1991) and Patterson et al. (under review) found a relatively rapid return of plasma volume to basal levels following a math stressor (10 minutes and 30 minutes, respectively), thus suggesting that the stress-induced mechanism for changes in plasma volume produces short-term effects such as fluid

shifting from the vascular space to the interstitial spaces and back again. However, Muldoon et al. (1992) found that plasma volume was still significantly decreased 30 minutes after the termination of a 20-minute computerized math-Stroop stressor, suggesting that the mechanism affected by stress can produce long-term changes in plasma volume such as increased water clearance by the kidney.

Unlike postural change, the mechanism or mechanisms for stress-mediated decreases in plasma volume have not yet been identified, and no data are available as to possible gender differences in the acute effects of psychological stress on plasma volume. The latter point is of particular interest because gender differences have been observed in stress-induced changes in cholesterol concentrations (Stoney, Matthews, McDonald, & Johnson, 1988). Stress-mediated changes in plasma volume have accounted for changes in lipids during acute stress in healthy men and it is possible that gender differences in plasma volume may explain gender differences in lipid concentrations.

Although the precise reasons for stress-induced decreases in plasma volume remain to be defined, plausible mechanisms can be suggested based on the known physiological effects of physical or psychological stress. Research on the effects of postural change (Hinghofer-Szalkay & Moser, 1986; Hinghofer-Szalkay & Greenleaf, 1987) and physical exercise (Smith, Guyton, Manning, & White, 1976; Nadel, Cafarelli, Roberts, &

Renger, 1979) indicate that decreased plasma volume is due to a transvascular shift of fluid from the intravascular space to the interstitial spaces by changes in hydrostatic or hemodynamic pressure. Stress-induced increases in red cell mass have also been implicated as a possible cause of increased hemoconcentration and decreased plasma volume (Cannon, 1932).

Accordingly, this dissertation research evaluated several possible mechanisms that could contribute to the decreases in plasma volume produced by psychological stress. This research consisted of studying, under controlled laboratory conditions, the time course of stress-induced plasma volume changes in order to determine whether changes in plasma volume are mediated by mechanisms that characteristically produce long-term (e.g., increased water clearance by the kidney) or short-term (e.g., transvascular shift) changes in plasma volume. This research also assessed the association of plasma volume changes over time with hemodynamic reactivity and changes in other hematologic factors during stress. Possible gender differences in changes in plasma volume were also evaluated by comparing these responses in men and women.

To provide a rationale for the research hypotheses, a review of research dealing with plasma volume regulation, the effects of psychological stress on hematologic variables, and possible mechanisms for plasma volume changes during stress is presented below.

The Importance of Plasma and Plasma Volume Regulation

Blood, which makes up about 6% of total body weight, is a suspension of various types of cells in an aqueous medium, the plasma. These cells serve multiple functions essential for metabolism and defense against injury. Healthy adults have an average of 20 ml to 40 ml of plasma per kilogram of body weight, totalling about 3 liters. Many substances are found in plasma, including proteins, electrolytes, lipids, amino acids, hormones, vitamins, carbohydrates, nitrogenous breakdown products of metabolism, and gaseous oxygen, nitrogen, and carbon dioxide. The ionic constituents of plasma maintain the pH of blood within physiological limits, while also maintaining the osmolality of plasma somewhere between 200 and 300 mOsm/kg water. The ionic equilibrium of plasma is maintained by the balance of inorganic cations (i.e., sodium, potassium, and calcium) and anions (i.e., chloride, bicarbonate, and plasma protein).

The role of plasma proteins. Within the plasma there are literally hundreds of different dissolved proteins totalling about 7 grams per deciliter (dl). The bulk of protein in the plasma is albumin, which is present at an average concentration of 4 g/dl. Because albumin diffuses poorly through the inner lining (endothelium) of intact blood vessels, it provides the critical colloid osmotic or oncotic pressure that regulates the passage of diffusible solutes and water through the capillaries. When the concentration of

albumin is severely reduced, excessive extracellular fluid may accumulate in the extravascular tissues and cause edema. Some of the other plasma proteins include antibodies that defend against infection, "clotting factors" needed for the coagulation of blood, and plasma lipids (e.g., triglycerides, cholesterol, or phospholipids).

Long-term plasma volume regulation. Because plasma is able to diffuse freely and rapidly across the precapillary and capillary beds whereas the various cells and proteins of the blood cannot, plasma volume plays an important role in the maintenance of blood volume homeostasis, especially during conditions of decreased blood volume or hypovolemia. However, vascular diffusibility is only a property of plasma, and plasma by itself is not able to control blood volume. Instead, it is the kidneys that are primarily responsible for long-term homeostasis. The kidneys have two main functions: 1) they excrete the primary end-products of bodily metabolism, and 2) they control the concentrations of most constituents of body fluids. Through these activities, the kidneys play a vital role in regulating the salt and water balance of the body. Although weighing only 300 grams, the kidney is a highly vascular organ which receives a blood flow of 1000 to 1200 ml/min, about 20% of the total cardiac output. The kidney regulates the composition of the extracellular fluid by selectively adjusting the composition of the glomerular filtrate through filtration, reabsorption, and secretion

(urea), and thus serves as the main regulator of the body's water balance.

Hormonal mechanisms of long-term plasma regulation. There are three neurohormonal mechanisms that can significantly influence renal blood flow, and thus regulate plasma volume. First is the renin-angiotensin-aldosterone (RAA) system that is concerned with the renal control of sodium. Increased release of renin by the kidney leads to a greater release of aldosterone through the angiotensin I-angiotensin II chain. An increase in aldosterone leads to an increase in sodium reabsorption by the renal distal tubules. The reabsorption of sodium carries water with it and thus increases plasma volume. The primary stimulus for renin secretion is increased sympathetic nervous system activity produced reflexly by decreased central blood volume or atrial pressure. A second mechanism is the antidiuretic hormone (ADH) system which is concerned with the renal control of free water. ADH is released from the posterior pituitary gland in response to a number of stimuli including: 1) activation of the sympathetic nervous system by emotion or decreased plasma volume as sensed by cardiopulmonary receptors; and 2) increased osmolality of the interstitial fluid of the brain (as sensed by osmoreceptors). ADH acts on the collecting tubules of the kidney to increase reabsorption of free water, inhibit diuresis, and increase plasma volume. The third mechanism is the release of atrial natriuretic peptide (ANP)

present in the atrial tissue which dilates renal vessels and influences water and electrolyte balance. ANP is released during distension of the atria and causes renal dilation, increased glomerular filtration, inhibition of sodium reabsorption, increased natriuresis, and a resultant reduction in extracellular fluid and plasma volume.

In reviewing the importance of plasma volume regulation, it is important to remember that the mechanisms described above are instrumental in the long-term maintenance of homeostasis, and not rapid responses to acute changes in plasma volume. The lack of renal control over acute changes in blood or plasma volume has been supported by several studies that have assessed the effects of acute psychological stress on renal function. In a study conducted by Fauvel et al (1991), renal glomerular filtration rate and renal plasma flow were unaltered by acute psychological stress. In this study, renal function and heart rate were monitored in fifteen healthy subjects (eight women and seven men) while preforming the Stroop color-word conflict task. Changes in renal function were determined by changes in glomerular filtration rate (inulin clearance) and renal plasma flow (para-aminohippurate clearance). Results indicated that, although there was a significant increase in heart rate and blood pressure, glomerular filtration rate and renal plasma flow during stress were unchanged.

Similar results were found in another study that assessed

the effects of stressful competition on urinary volume and sodium excretion in healthy male college students (Light, Keopke, Obrist, & Willis, 1983). Subjects in this study were grouped on the basis of 1) whether their parents were normotensive, or at least one parent was hypertensive; and 2) whether they responded to the competitive task with little change or with a large increase in heart rate. All subjects attended a five-hour study session in which the first 3 hours were used to establish regular urinary voiding, while the fourth hour served as the stress period and the fifth hour served as a recovery period. Light et al. found that the only group to exhibit any significant change in sodium or fluid excretion was the one of subjects with a positive family history of hypertension who were also high heart rate reactors. The findings of this study suggest that psychological stress can cause a significant increase in hemodynamic reactivity with no effect on kidney sodium or fluid excretion in normal healthy individuals, whereas increased sodium and fluid retention appears more likely in individuals at high risk for hypertension. Thus, the outcomes of both these studies suggest that although psychological stress produces changes in hemodynamic reactivity in healthy individuals, renal function, in terms of glomerular plasma filtration and fluid excretion, are not affected.

In sum, plasma is one of the main constituents of blood, accounting for about 55% of the total blood volume. Plasma

functions as the fluid medium in which the blood cells and protein nutrients are carried throughout the body. Long-term plasma volume regulation is maintained primarily by the kidney during normal blood volume homeostasis. However, research examining the effects of psychological stress on physiological processes in the body have found that stress can have an acute effect on several hematological factors.

Stress and Stress Effects on Blood and Blood Constituents

The term "stress" has been widely and indiscriminantly used. However, for purposes of this dissertation, stress is "the process by which environmental events threaten or challenge an organism's well-being and by which the organism responds to this threat" (Gatchel, Baum, & Krantz, 1988). In this definition, environmental events are regarded as uncontrollable events or stressors that are perceived by an individual as threatening to and capable of producing physical or psychological harm. Physical harm can range from the discomfort of submerging a hand in ice water to the gross bodily harm of being shot and wounded. Psychological harm can include damage to self-esteem, impoverished environments, or the disruption of social relationships. Stress includes a set of responses elicited by the perception of a stressor as harmful or threatening and these stress responses range from physiological changes (e.g., increased sympathoadrenergic activation), to psychological (e.g., feelings of anger, fear,

or anxiety), and behavioral changes (e.g., coping). Although there is still a great deal yet to be learned about stress, past research by individuals such as Cannon and Selye has revealed important facts and relationships that help to interpret the source, characteristics, and effects of stress on the human body.

Hans Selye (1936, 1967) is the individual most often associated with the term stress. However, Walter Cannon (1914) used the term nearly a quarter of a century before Selye and was one of the first researchers to clearly suggest that the stress process involves both physiological and psychological components. Most of Cannon's work assessed stress-mediated physiological changes in animals, and viewed the emotional stress as a potential cause of medical problems due to the effects of stress on autonomic nervous system activity. According to Cannon (1929), the parasympathetic nervous system had the role of "conserver of bodily energies", and the sympathetic nervous system had an "emergency" function. Cannon stated that by temporarily shifting the autonomic balance toward sympathicotonia (increased tonus of the sympathetic system) the organism prepares for a quick release of energy for the purpose of "fight or flight" and thereby reduces the restitutive processes. During the "fight or flight" response associated with sympathetic excitation, widespread changes occur such as increased sweating, increased heart rate, and a redistribution of blood from the gut to the

muscles. Additional adrenergic changes associated with stress included liberation of glucose into the bloodstream for increased energy, improved contraction of fatigued muscles, and more rapid coagulation of the blood in case of injury. Cannon (1929) felt that all of these sympathetically-mediated responses allowed the organism to mobilize for prompt and efficient action in response to threat or danger and to withstand and defeat that danger. His concept that aversive stimuli produce a global activation of the sympathetic nervous system had a great impact on later psychologists seeking a unified explanation of the stress response.

Cannon (1929) was also one of the first researchers to identify a relationship between sympathetic nervous system activation and various hematologic and hemostatic processes. Cannon found that during periods of emotional upset in cats, such as anger or rage produced by the presentation of large dogs, sympathetic activity increased and subsequently, heart rate, blood pressure, and red blood cell (RBC) counts increased as well. He argued that this hemodynamic reactivity and increase in circulating RBC's during emotional arousal was adaptive to survival because it increased the amount of oxygen in the blood that could be circulated and utilized by muscles needed for defense, such as the muscles of the arms and legs.

Hans Selye's concept of stress refers to a characteristic physiological response, differentiating this from "stressors"-

- harmful or noxious agents which produce stress (1950). Selye argued that the stress response consisted of a non-specific General Adaptation Syndrome (GAS) and that any stressor triggered the same pattern of responses. When stress is prolonged, the GAS can include three stages: 1) an alarm reaction, including the initial shock phase of lowered resistance to the stressor and a countershock phase, in which corticosteroids are released and defensive mechanisms begin to operate; 2) a stage of resistance in which adaptation is optimal; and 3) a stage of exhaustion, marked by the collapse of the body's adaptive response and decreased resistance to other stimuli.

Selye's GAS emphasizes responses of the pituitary-adrenal cortical axis. Selye described the results of the GAS reaction as a triad of responses consisting of enlargement of the adrenals, shrinkage of the thymus and lymph nodes, and gastrointestinal ulceration. His model of the General Adaptation Syndrome is probably most notable for its description of how long term stress leads to resistance and possible physiological damage.

Much like Cannon's observations of stress effects on hematologic factors, Selye found similar changes in the numbers of blood cells during psychological stress. During his study of the various components involved in his GAS model (1936, 1950), Selye discovered that the presentation of almost any noxious stimulus (e.g., injected albumin, exposure to x-

ray radiation, heat and cold exposure) produced an increase in not only red blood cells, but also a significant increase in white blood cells. Selye postulated that the increase in white blood cells reflected an organism's attempt at strengthening its immunity to various stressors during the alarm reaction and resistance stage.

In sum, our current conceptualization of the stress response is the product of a long evolutionary process that began with Cannon's concept of the global sympathetic "fight or flight" response and Selye's General Adaptation Syndrome theory of stress responding. The culmination of research by these and many other researchers has lead to our understanding that stress is a "negative emotional experience accompanied by predictable biochemical, physiological, and behavioral changes that are directed toward adaptation either by manipulating the situation to alter the stressor or by accommodating its effects", (Baum, 1990). Among these predictable physiological changes is one element that has been recognized since the early stress response work of Cannon: stress-induced changes in hematologic variables.

Recent Research on Hematologic Responses to Stress

Since early research on the stress response, the concept and experimental analysis of stress has undergone dynamic change. Most notably, current technology has made it possible to describe more precisely the processes involved in human

stress experiences. However, it is interesting to note that from the time of Cannon's research on the "fight or flight" response to the present, one stress-mediated physiological response that has been observed repeatedly, but has received little attention, is change in the various constituents of the blood.

Recently however, interest in stress and hematology has increased as researchers have found that acute psychological stress not only leads to an increase in red and white blood cells, but can also lead to alterations in several other constituents of the blood that are related to coronary artery disease (e.g., platelets, fibrinogen, lipids). In a study designed to examine the hematological effect of emotional stress in healthy men, a significant increase in peripheral blood cells, blood pressure, and heart rate was found during the performance of a ten-minute mental arithmetic task (Jern et al, 1988). Jern et al also found that an increase in RBC count was significantly related to heart rate reactivity, whereas the increase in WBC count correlated with increased blood pressure. In a follow-up study using multiple stressors to assess the effects of stress on plasma coagulation, it was observed that heart rate, blood pressure, and fibrinogen concentrations increased significantly during a 20 minute psychological stress period consisting of a ten-minute Stroop color-word test followed by a 10-minute mental arithmetic task (Jern, Eriksson, Tengborn, Risberg, Wadenvik, & Jern, 1989.

However, no significant correlations were found between changes in fibrinogen concentrations and hemodynamic variables.

As with studies of blood cell reactivity to stress, several studies have also been conducted to assess the effects of acute psychological stress on lipid concentrations in the blood. Although lipids are not cellular components of the blood, they are large blood-borne particles that account for a large portion of the proteins found in the blood and are believed to play an integral role in the pathogenesis of cardiovascular disease (Kannel, Castelli, & Gordon, 1979). Stoney et al. (1988) assessed the effects of various stressors (mental arithmetic, speech task, and isometric exercise) on LDL-C, HDL-C, triglycerides, and free fatty acid in men and women. Besides a significant increase in blood pressure and heart rate, their results revealed that LDL increased significantly during all tasks in both men and women, with men having the greatest blood pressure and LDL increases during each task. Similar results were found in another study assessing gender differences in lipid reactivity to mirror tracing and Stroop test stress (Matthews, Davis, Stoney, Owen, & Caggiula, 1991). Matthews et al found an overall increase in cholesterol, LDL-C, and HDL-C during the mirror tracing task in men and women. An overall increase in cholesterol, LDL-C, HDL-C, triglycerides, and free fatty acids was also found during a 10-minute Stroop test, with men showing greater

changes in LDL-C and blood pressure than women during the test. Stress-mediated increases in cholesterol have also been shown to be related to changes in blood pressure. In a study of 106 healthy male college students, Gillum, Taylor, Anderson, & Blackburn (1981) found that absolute levels of systolic blood pressure and changes from baseline during a one-minute cold pressor test correlated positively with total cholesterol.

In summary, research over the years has demonstrated repeatedly that most of the blood constituents (e.g., RBC's, WBC's, platelets, fibrinogen, cholesterol) are affected by acute psychological stress. Interestingly, the effect of stress on these blood constituents has been strikingly similar in that stress produces an increase in each of these variables, rather than a decrease. This point becomes increasingly important in the following section since changes in plasma volume can have direct effects on the concentration of the other constituents of the blood.

Possible Effects of Alterations in Plasma Volume

Recently another component of the blood, plasma, has been found to be acutely affected by psychological stress. Changes in plasma volume due to transvascular fluid shifts, as seen during postural changes (see below) (Tan, Wilmshurst, Gleason, & Soeldner, 1973), lead to hemoconcentration, or a relative increase in blood cells and proteins resulting from decreased

plasma volume. Since large blood constituents cannot freely pass through the small slit-like pores or clefts (averaging about 40 Angstroms in width) between the endothelial cells, the concentration of the non-diffusible blood constituents increases when fluid diffuses across the blood vessel wall. For example, acute increases in plasma volume can lead to hemodilution of blood cells and large protein particles, whereas an acute decrease in plasma volume can lead to hemoconcentration of cells and proteins. Thus, it is possible that what has been viewed as stress-mediated increases in blood cells and proteins released from potential stores in the spleen or liver, may actually be secondary to decreases in plasma volume.

Effects of Posture on Plasma Volume

Although psychological stress-mediated decreases in plasma volume have only recently been observed, acute decreases in plasma volume have long been observed during changes in posture, from sitting to standing, and was the impetus for studies of the effects of psychological stress on plasma volume. As early as the late 1920's, it was recognized that posture had an effect on the composition and volume of plasma. Early research found that hematocrit levels increased by as much as 14% when subjects went from a seated to standing position and returned to normal levels after subjects resumed a seated position (Thompson et al, 1929; Waterfield, 1931).

This postural effect on plasma volume was later found to be due to increased hydrostatic pressure in the lower extremities caused by assuming an upright posture. Initially, within the first 10 to 15 seconds following a change from a seated or recumbent position to standing, cardiac output and stroke volume decrease by about 20%. Along with this drop in cardiac output and stroke volume, blood pressure briefly decreases and heart rate increases producing momentary hypotension, while venous pooling of blood begins to increase in the lower extremities. Following the initial hypotension (10 to 15 seconds), blood pressure begins to steadily increase to accommodate to the change in blood distribution in the body (Smith & Kampine, 1990). Youmans, Wells, Donley, Miller, and Frank (1934) demonstrated that by changing posture from sitting to standing, intravascular plasma volume decreased and interstitial fluid levels increased until the colloid osmotic pressure of plasma protein concentrations equilibrated with that of the interstitial space fluid pressure. Although these early studies were among the first to show the effects of posture effects on intravascular plasma levels, the measures used to estimate plasma volume from hematocrit were crude and subject to error due to plasma trapped in the packed cell volume.

Effects of Psychological Stress on Plasma Volume

Recently, research on the physiological effects of

psychological stress has found similar changes in plasma volume during acute stress. However, unlike that of posture-mediated plasma volume changes, the mechanisms for stress-induced decreases in plasma volume are not well defined.

In a study conducted to determine the importance of emotional stress on hematocrit and hemoglobin levels (Jern, Jern, & Wadenvik, 1991), hematologic and hemodynamic factors were assessed in 11 subjects with the Type A behavior pattern and 11 Type B subjects during a ten-minute mental arithmetic task without harassment. Results indicated that both groups showed a significant increase in hematocrit and hemoglobin levels during stress and that stress-induced hemoconcentration was not significantly different between the A- and B-groups. Although specific mechanisms were not addressed in this study, a significant correlation was found between resting diastolic blood pressure and hemoglobin concentration ($r=0.53$) for the whole group, suggesting possible ^Phemodynamic influence over hemoconcentration. Jern et al. also reported that hematocrit and hemoglobin levels had returned to baseline within 10 minutes following stressor termination.

Another study by Muldoon et al. (1992) found that stress-mediated increases in circulating serum cholesterol concentrations during presentation of an acute psychological stressor could be accounted for by significant decreases in plasma volume. In this study, male subjects attended two laboratory sessions, each consisting of a baseline (30

minutes), task (20 minutes), and recovery (30 minutes) period, in order to assess the effects of two different tasks on serum cholesterol and plasma volume. During the task period of one session, subjects performed a mental task (computerized Stroop test and mental arithmetic task); during the second session, subjects stood erect during the task period. Muldoon et al found that serum cholesterol concentrations increased significantly during both the psychological stress task and the posture task (3.7 and 21.9 mg/dl, respectively). However, Muldoon et al. also found that plasma volume decreased significantly during both tasks. By correcting for the hemoconcentration of large particle substances, such as serum cholesterol, produced by deceased plasma volume, stress effects on cholesterol levels were no longer evident. The authors also noted that the changes in plasma volume remained significantly lower throughout the recovery period, and thus were unable to identify any plausible mechanisms for the plasma change, but suggested increased kidney function and diuresis as a possible cause due to the long duration of the plasma volume change.

In a more recent study by Patterson et al. (in press), it was observed that psychological stress produced acute decreases in plasma volume and an increase in lipid and protein concentrations. Changes in estimated plasma volume was assessed in 18 healthy, normotensive males while performing a 10 minute mental arithmetic task. An additional

5 subjects served as controls. Subjects attended a laboratory session consisting of a 30-minute rest period, 10-minute mental arithmetic task with harassment, and a 30-minute recovery period. Estimated plasma volume was arithmetically calculated using hematocrit and hemoglobin values following the method described by Dill and Costill (1974). The results indicated a significant increase in both hematocrit and hemoglobin during the mental arithmetic task which corresponded to a 9.23% decrease in plasma volume, while no changes were observed in the control group. Similar to the hemoconcentration results reported by Muldoon et al. (1992), Patterson et al. (in press) found that the decrease in plasma volume led to a hemoconcentration of cholesterol, triglycerides, low-density lipoprotein, high-density lipoprotein, and total plasma protein. However, unlike that of Muldoon et al, this study found that hematocrit and hemoglobin levels returned to baseline within 30 minutes of the stressor termination. This rapid recovery of plasma volume to basal levels indicates that the mechanism for stress-induced changes in plasma volume facilitates not only a rapid decrease in plasma volume during psychological stress but also a rapid recovery once the stressor is terminated, thus indicating a mechanism that would allow for rapid fluid shifts such as transvascular fluid movement. A potential transvascular fluid shift mechanism has been further supported by data (Patterson et al, under review), demonstrating a

significant decrease in plasma volume during a 2½ minute cold pressor test and during a mental arithmetic task. These changes in plasma volume were also found to be negatively associated with changes in mean arterial pressure ($r=-0.67$ and $r=-0.49$, respectively), which is consistent with hemodynamic effects on transvascular fluid shifts during postural changes.

Although actual mechanisms for the stress-induced plasma volume changes such as transvascular fluid movement or increased kidney function were not assessed in the above mentioned studies, it appears that the mechanism or mechanisms would have to facilitate rapid fluid exchange based on the relationships found between blood pressure and plasma volume (Jern et al, 1991; Patterson et al, under review) and the rapid return of plasma volume to baseline levels following stressor termination (Jern et al, 1991; Patterson et al, in press), thus ruling out the possibility of a more long-term mechanism such as kidney function (Muldoon et al, 1992). Increased kidney function can be further ruled out by the previously mentioned studies that directly assessed kidney function during stress and found little or no changes in kidney function during acute stress (Fauvel et al, 1991; Light et al, 1983).

Gender Differences in Hemodynamic, Lipid, and Hematologic Responses During Stress

Possible gender differences in stress-mediated decreases in plasma volume have not yet been investigated. However,

research on gender differences during psychological stress has found that men and women differ on physiological variables that are either associated with changes in plasma volume (e.g., blood pressure; Patterson et al, under review) or that are affected by changes in plasma volume (e.g., lipids; Patterson et al, in press; Muldoon et al, 1992). The only literature that has directly addressed gender differences in plasma volume changes has been in the area of physical exercise. The evidence for gender differences in each of these areas (hemodynamic reactivity, lipid changes, and physical exercise) will be reviewed in detail below.

Hemodynamic reactivity. A few studies have assessed gender differences in hemodynamic responses during psychological stress. In terms of blood pressure responses to behavioral challenges, such as cognitive and psychomotor tasks, several investigators have demonstrated that adult males exhibit greater systolic blood pressure responses relative to females of the same age (Matthews et al, 1991; Matthews & Stoney, 1988; Lundberg, Fredrikson, Wallin, Melin, & Frankenhaeuser, 1989). Other investigators, however, did not observe gender differences in blood pressure during behavioral challenge (Girdler, Turner, Sherwood, & Light, 1990; Jorgensen & Houston, 1981). In the study reported by Girdler et al. (1990), the hemodynamic mechanism mediating pressor responses during several different stressors (computerized math task, serial subtraction, interpersonal

speech task, verbalization of content speech task) was different for males and females. They found that females exhibited increases in blood pressure through greater myocardial reactivity (cardiac output) relative to males, while males showed increases in blood pressure through more enhanced vascular reactivity (peripheral resistance). Similarly, although Jorgensen and Houston (1981) failed to find gender differences in hemodynamic reactivity during mental arithmetic and cognitive-conflict tasks, they did observe greater systolic blood pressure elevations in men during the recovery period following the stressors relative to women.

Lipids and lipoproteins. As mentioned earlier, it is interesting to note that gender differences have been observed in stress-mediated increases in lipid concentrations, with men having larger increases in lipids during stress than women (van Doornen, 1986; Stoney et al, 1988; Matthews et al, 1991). Stoney et al. (1988) examined whether or not lipoproteins and lipids changed in response to behavioral stress (mental arithmetic, speech task, and isometric exercise) in men and women, and the extent of gender differences in lipid, neuroendocrine, and cardiovascular changes during stress. They found significant increases in blood pressure, heart rate, and LDL-C during all tasks in both men and women. In subsequent analyses of gender differences, Stoney et al. found that males had larger increases in LDL-C, and triglycerides

during each task. Similar gender differences in lipid concentrations were found by Matthews et al. (1991) during mirror tracing and Stroop tasks. Matthews et al. found an overall increase in cholesterol, LDL-C, HDL-C, triglycerides, and free fatty acids during both tasks. However, significant gender differences were found in LDL-C concentrations during the Stroop test, with men showing greater changes in LDL-C than women during the test.

It is interesting to note that in each of the studies that reported higher lipoprotein concentrations in men versus women during stress, men also exhibited larger blood pressure responses. This observation is further supported by a study that examined the relationship between lipids and cardiovascular function during different field and laboratory conditions in men and women (Lundberg et al, 1989). Lundberg et al. assessed the relationship between fasting lipids (serum cholesterol, HDL-C, LDL-C, triglycerides) and repeated measurements of systolic and diastolic blood pressure and heart rate under the following conditions: 1) a day at work, 2) a day at home, 3) laboratory stressors (star-tracing, mental arithmetic, Stroop, cold pressor, hand-grip and a Type A interview), and 4) laboratory rest. The results indicated that in men, but not in women, there was a reliable positive relationship between systolic blood pressure and serum cholesterol, LDL-C, and HDL-C in all conditions. Although it is speculative at this point, it is possible that there are

gender differences in transvascular fluid shifts due to differences in hemodynamic reactivity, and that this may account for gender differences in lipid concentrations based on differences in hemoconcentration. However, as stated previously, no investigators have addressed this possibility.

Physical exercise. The only studies addressing the issue of gender differences in plasma volume responses to environmental stimuli have examined heat and exercise. Studies in this area have reported that blood volume in women is lower than that of men when expressed either in absolute terms or in terms of unit body weight. However, within both sexes, deviations from normal can be traced to differences in adiposity, and this accounts for the lower values per unit weight in women (Gregerson & Rawson, 1959). When lean body weights are compared, these differences disappear.

Although the extra subcutaneous fat in women confers obvious advantages in the cold, its effect on response to thermal stress is slight because heat transport is dependent only upon the cardiovascular system and is independent of skin fold thickness (Bernstein, Johnson, Ryan, Inouye, & Hick, 1956). On the other hand, the capacity for dissipation of transported heat is dependent upon the surface area available for heat exchange with the environment. Women have a greater surface-to-mass ratio than men (Nunneley, 1979), based on their total- or lean-body weight. In the case of the latter the difference is considerable; in young adults it can amount

to 25% (MacMillan, Reid, Shirling, & Passmore, 1965). Because volume is also smaller, a proportionally greater fraction of the total blood volume is required to perfuse the skin (assuming a similar distribution of cutaneous blood vessels). Consequently, a greater portion of plasma would be lost to the interstitial spaces during heat exposure, and women would therefore hemoconcentrate more in the heat than males; according to Senay (1973) in this case. He found that women, in contrast to men (Senay & Christensen, 1965), exhibited greater hemoconcentration when exposed to heat (Senay & Fortney, 1975) and during exercise (Senay, 1972; Senay & Kok, 1977). However, in Senay's exercise studies, bench stepping was used for men, and the bicycle ergometer, which usually produces the greatest hemoconcentration, for the women. Hence, mode of exercise, rather than sex differences, may explain the discrepancies between male and female intravascular volume responses to exercise.

Senay's findings are contrary to those of other researchers (Wells & Horvath, 1973; Drinkwater, Denton, Kupprat, Talag, & Horvath, 1976), who have observed hemodilution in women at rest in a hot environment and no hemoconcentration in women during exercise in the heat, the latter of which is consistent with observations of men exercising in the heat. More recent studies (Sawka, Toner, Francesconi, & Pandolf, 1983; Sawka, Francesconi, Pimental, & Pandolf, 1984) with subjects matched for fitness level have

failed to detect any effect of gender on physiological responses, including intravascular volume responses. Thus, there is currently little convincing evidence that intravascular plasma volume responses to thermal or exercise stress differ significantly between males and females, and no studies exist regarding possible gender differences in plasma volume changes during acute psychological stress.

Menstrual cycle effects on physiologic responses to stress. Studies examining the effects of the different phases of the menstrual cycle on cardiovascular reactivity and body-fluid shifts have produced inconsistent results. Polefrone and Manuck (1988) found that cardiovascular reactivity during a difficult "concept-forming abilities" test and a mental arithmetic task was greatest during the follicular phase (day 17-21) when compared to the luteal phase (day 7-11). Opposite results were found by Hastrup and Light (1984) in that women in the luteal phase had greater heart rate and blood pressure changes during a shock avoidance task than women in the follicular phase. Other investigators have found no differences in cardiovascular reactivity as a function of the different phases of the menstrual cycle (Strauss, Schultheiss, & Cohen, 1983; Carroll, Turner, Lee, & Stephenson, 1984). Conflicting evidence also exists with regard to menstrual cycle effects on plasma volume changes in females during either heat exposure or physical exercise. While studies by Gaebelein and Senay (1982) found that during heat exposure and

physical exercise there was a tendency toward decreased plasma volume during the follicular phase rather than in the luteal phase, other studies have found minimal differences between the follicular and luteal phases in fluid shifts during heat exposure (Wells & Horvath, 1973) or exercise (Wells & Horvath, 1974). Although there appear to be few reliable menstrual cycle effects on cardiovascular and hematologic reactivity during physical or psychological stress, it is still important methodologically to establish consistent indexing of subject's menstrual phase and to study female subjects at comparable points in the menstrual cycle.

Possible Mechanisms for Acute Stress-Induced Contracted Plasma Volume

It is possible to suggest several physiological mechanisms to account for stress-mediated changes in plasma volume. However, before discussing these mechanisms, it is necessary to describe the various compartments of the body in which fluid can be found in order to understand where shifts in fluid can take place.

Total body water comprises about 60% of the body mass in a normal subject and is distributed among the different fluid compartments that are generally categorized as either intracellular or extracellular. Tissues such as fat and bone contain little water, and since the volumes of the various compartments are expressed as a percentage of body weight, it is important to note that increases in the quantity of either

fat or bone can decrease the fractional content of body water. As previously stated, body water is partitioned between intracellular fluid, which is 33% of the body weight, and extracellular fluid, which is 27% of body weight. The water concentration differences between the intracellular and extracellular compartments is largely influenced by the cell membranes separating them.

Extracellular space is a combination of three main volumes that are functionally different. The first volume is the interstitial fluid which is the fluid in intimate contact with the cells of the body and represents about 12% of body weight. The second volume is the liquid medium of the blood, called plasma, which transports the formed cellular elements and proteins of the blood. Plasma represents 4.5% of body weight, and together with interstitial fluid make up the portion of extracellular fluid that can rapidly equilibrate in various parts of the body if necessary. The third fraction of extracellular fluid is relatively inaccessible, since it is bound up in the matrices of bone and cartilage or contained in closed cavities such as the cerebral ventricles of the brain or joints (transcellular water). This inaccessible fraction amounts to about 10.5% of the body weight. Thus, the total extracellular volume (27% of body weight) is the sum of the bound fluid, plasma volume, and the interstitial fluid. The intracellular fluid, on the other hand, is comprised of all the fluid found in the cells of the body, to include the red

blood cells which account for approximately 2.5% of body weight.

Given that fluid can freely pass between several of the above mentioned compartments, several possible mechanisms for the stress-induced plasma volume changes become evident and will be described in some detail.

Transvascular fluid movement. One possible mechanism that may be responsible for stress-mediated decreases in plasma volume is an increase in transvascular fluid movement across the vascular endothelium and into the interstitial space due to increased arterial pressure (see Figure 1a). As mentioned earlier, this has been recognized for many years as the mechanism for decreases in plasma volume and increases in plasma protein concentrations in the blood during changes in posture. When an individual goes from a seated position to a standing position, gravitational forces draw blood down to the lower extremities. As the hydrostatic pressure in the lower extremities increases, all substances that are diffusible across the vascular membranes (e.g., water, sodium, albumin) will exit the vasculature into the interstitial space until the oncotic pressure in the interstitial space is equal to the intravascular colloid osmotic pressure produced by decreased plasma volume and an increase in non-diffusible substances in the blood (e.g., cells, fibrinogen, cholesterol).

This mechanism for the rapid fluid shifts is also supported by clinical observations of a condition in which

increased blood pressure has been found to be associated with decreased plasma volume. Although hypertension is a chronic condition that is distinguished by a chronic elevation in blood pressure, studies examining "stress" or relative polycythemia (characterized by normal red cell mass and decreased plasma volume) have found that relative polycythemia is associated with hypertension (Bing & Smith, 1981; Korbin, Frohlich, & Ventura, 1984) and that treatment of hypertension with antihypertensive therapy results in plasma volume expansion to near normal levels (Finnerty, Buchholz, & Guillaudeu, 1958; Emory, Whitcomb, & Frohlich, 1974). Relative polycythemia is a distinct and commonly encountered condition that is also referred to as spurious polycythemia (Weinreb & Shih, 1975), Gaisbock's disease (Russell & Conely, 1964; Hall, 1965), and stress erythrocytosis (Dameshek, 1953). It is not a primary disease process and may be merely a physiological state in which the plasma volume is slightly reduced and the red cell mass is unchanged, and other blood constituents are normal. Hematocrit is elevated, but rarely exceeds 60 percent. Hence, it is possible that acute alterations in blood pressure could produce systemic changes in plasma volume due to changes in intravascular pressure.

Increased red cell mass. A second possible cause for an apparent reduction in plasma volume is an increase in peripheral red cell mass (RCM) (see Figure 1b). It should be

noted that the term "apparent reduction" is used rather than "actual reduction" in describing this potential mechanism. This is because the appearance of decreased plasma volume in this condition is actually due to an absolute increase in red blood cells and subsequent hypervolemia which gives rise to increased hematocrit with no change in plasma volume, and thus is not a decrease in plasma volume at all. A clinical example of this phenomenon would be a condition called polycythemia, meaning "many cells". Polycythemia is an increase in red blood cell production or erythrocytosis with an increase in both hemoglobin concentration and packed cell volume (hematocrit). Hemoglobin, hematocrit and red cell count are parameters that are measured in relative terms (e.g., the ratio of hemoglobin or erythrocytes to plasma volume), not in absolute concentrations. Two forms of polycythemia are polycythemia vera and secondary polycythemia, both of which are results of an absolute increase in the total body red cell mass and no change in plasma volume (McKenzie, 1988). Polycythemia vera is caused by a primary unregulated increase in red cell production, with an insidious onset, chronic course, and no identifiable inciting cause (Athens, 1993). Secondary polycythemia, on the other hand, can be distinguished from polycythemia vera by a distinct, although not always apparent, explanation for the erythrocytosis such as high altitude hypoxia (Erslev, 1980), or exposure to carbon monoxide (Bartlett, 1968; Isager & Hagerup, 1971). Hence the

name "secondary" polycythemia.

Although the term "red cell mass" is usually used to describe the total number of red blood cells in the body (Grable & Williams, 1968) as in the conditions of acute mountain sickness or secondary polycythemia (Hackett, Rennie, & Levine, 1976) and polycythemia vera (Wasserman & Gilbert, 1966) described above, the term here will be used to describe potential changes in peripheral red cell mass due to the sequestration of cells from blood reservoirs in the body. The sequestering of blood cells into the main circulation is usually caused by sympathetic vasoconstriction, and the extent of vasoconstriction is more prominent in certain areas of the body than in others. The vascular bed of the skin is one of the major blood reservoirs in humans. For example, during blood loss, considerable subcutaneous vasoconstriction occurs, which leads to the characteristic pale appearance of the skin. This redistribution of blood away from the skin allows for the profusion of more vital areas with several hundred milliliters of blood. Although not as pronounced as subcutaneous vasoconstriction during hemorrhage, psychological stress can also cause vasoconstriction. Williams, Bittker, Buchsbaum, and Wynne (1975) clearly demonstrated that several different types of stressors (e.g., word recognition, mental arithmetic, and personal interview) all produced significant decreases in peripheral blood flow due to increased vasoconstriction in healthy individuals. However, in terms of increased RCM, the

composition of the blood (i.e., cells, plasma, proteins) redistributed by subcutaneous vasoconstriction is proportional to that in the main circulation. Thus, subcutaneous vasoconstriction alone would have little effect if any on peripheral red cell mass.

The vascular beds of the spleen and liver are also important blood reservoirs that are under sympathetic innervation. Cannon (1929) demonstrated that during periods of induced anger or fear, the canine spleen contracted and expelled up to 300 ml of red blood cells into the main circulation. This phenomenon was replicated by Barcroft (1929), who also demonstrated by x-ray imaging that the canine spleen can contract to as little as one-fifth its original size during emotional arousal. However, although in certain animals the spleen acts as an important reservoir of red blood cells that can be quickly released by sympathetic arousal, it has no such function in the human for the adult human spleen only holds about 30 to 40 ml of red cells. Moreover the human splenic capsule does not contain muscle cells and it can undergo little expansion or contraction (Ebert & Stead, 1941; Crosby, 1959). Only during enlargement of the spleen or splenomegaly is there a pronounced sequestration of red blood cells from the spleen in humans, often with profound effects on the peripheral cell counts (Jendl, Jacob, & Daland, 1961). The liver, on the other hand, contains a large reservoir of blood that can be rapidly released into circulation. The

normal blood volume of the human liver, including the hepatic veins and sinuses, is 450 milliliters, or almost 10% of the total blood volume (Guyton, 1991). Although no studies have addressed the effects of environpsychological stress on the expulsion of blood from the liver, several studies have demonstrated that direct stimulation of the hepatic nerves results in a rapid and well maintained decrease of up to 50% of the total liver blood volume in dogs and cats (Greenway & Stark, 1971). Similarly, research has shown that infusion of physiologic quantities of epinephrine (50 μg/l) into the hepatic artery of dogs produces marked hepatic artery constriction and an active expulsion of 71 ml/kg of blood from the liver (Bennett, MacAnespie, & Rothe, 1982).

Transcellular fluid movement. A final mechanism that warrants mention, in terms of plasma volume alteration, is osmotic swelling of the red blood cells or a transcellular influx of plasma fluid (see Figure 1c). This is distinguishable from transvascular fluid movement in that rather than fluid crossing the blood vessel walls into the interstitial space, fluid crosses the cellular membrane of blood cells in circulation. The plasma membranes of the red blood cells are relatively impermeable to many of the solutes in the blood but are highly permeable to water. Therefore, when the osmotic pressure of plasma is increased, water leaves the cells by osmosis, the cell shrinks, and the cellular

solute becomes more concentrated until the effective osmotic pressure of the cytoplasm is equal to that of the plasma. Conversely, if the osmotic pressure of the plasma is decreased, water enters the cells, and the cells will swell until the intracellular and extracellular osmotic pressures equilibrate (Berne & Levy, 1983). Since red blood cells can act as little "osmometers" in the circulation, it is possible that during the initial period of increased oncotic pressure produced by stress-mediated increases in blood pressure, plasma osmolarity decreases and leads to a transcellular influx of water which, in turn, produces a decrease in plasma volume. However, while this issue has not yet been addressed in research examining psychological stress effects on plasma volume, research on the effects of posture have found that increases in hydrostatic pressure have lead to either an increase in plasma osmolarity, which would decrease cellular fluid volume (Greenleaf, Convertino, & Mangseth, 1979), or no change in plasma osmolality (Hinghofer-Szalkay & Moser, 1986). Therefore, based on the effect of physical challenge on plasma osmolarity, it is likely that psychological stress would have little if any effect on plasma osmolarity, and thus leaning away from the possibility of cellular osmotic swelling accounting for stress-mediated decreases in plasma volume.

Method for Assessing Mechanisms of Stress-induced Decreased Plasma Volume

The different methods for assessing the potential

mechanisms for decreased plasma volume described above (transvascular fluid shift, increased red cell mass, and transcellular fluid shift) have each been well established as valuable clinical or research tools. Due to its simplicity, the method for assessing transcellular fluid shifts will be described first. Since the assessment of changes in both transvascular fluid shifts and red cell mass use the same techniques, the remainder of this section will describe in detail two currently used methods, as well as the shortcomings of each.

Method of transcellular fluid shift measurement. The assessment of potential transcellular fluid shifts is fairly simple and relies on standard detection techniques used in all clinical hematology laboratories. Since an influx or efflux of cellular fluid (transcellular fluid movement) produces either red blood cell swelling or crenation, the measurement of red blood cell size or mean corpuscular volume (MCV) is the standard technique for changes in transcellular fluid movement. The average volume of RBC's can be calculated from RBC count, which measures the number of cells per microliter of blood, and the packed cell volume, which measures the proportion of the blood occupied by the RBC's. The MCV can be measured by electric cell counters (Coulter cell counter) and dividing the summation of the cell volumes by the RBC count. Thus an increase in MCV would indicate an increase in the influx of fluid from plasma into blood cells and a decrease in

MCV indicates a efflux of cellular fluid into the vascular space.

Transvascular shift and red cell mass assessment techniques. As mentioned earlier, the main distinction between changes in plasma due to increased transvascular fluid shift and increased red cell mass is that increased transvascular fluid shift produces a hemoconcentration of non-diffusible proteins in the plasma, while increased red cell mass is characterized by an increase in RBC's with no appreciable effect on plasma protein concentrations. Two reliable techniques that can be utilized to assess changes in these mechanisms are nuclear medicine techniques using radioisotope-labeled blood cells and proteins and mass densitometry techniques.

Nuclear medicine techniques. With the advent of nuclear medicine, and its usefulness as a diagnostic tool in disease assessment, came more accurate techniques for the measurement of plasma volume. The most widely used nuclear medicine technique for the assessment of changes in plasma volume and the mechanism for the change is a method that uses chromium 51 (^{51}Cr) labeled RBC's for the measurement of the red cell mass and iodinated ^{125}I serum albumin for the measurement of the plasma component (Eckelman, 1975). Iodinated ^{125}I serum albumin is used to measure the plasma component because serum albumin is the largest protein particle in plasma that can passively cross through the pores in the vascular endothelium.

Together these two labeling techniques allow for very accurate measurement of total blood and plasma volume in the body. In using this technique, an decrease in chromium labeled RBC counts with no change in iodinated serum albumin counts would indicate an increase in red cell mass. Conversely, increased iodinated serum albumin counts with an increase in labeled RBC counts would indicate an increase in transvascular fluid shift from the intravascular space into the interstitial space. It is also possible for no increase in labeled RBC and albumin counts to occur which would indicate that red cell mass and plasma volume did not change. Finally, an increase in iodinated serum albumin counts with little or no change in labeled RBC counts would indicate that both transvascular fluid shifts and increased red cell mass are occurring simultaneously.

Although the isotope-labeling technique is most often used in the clinical diagnosis of blood volume disorders such as polycythemia vera or erythrocythemia, some researchers have used it to assess changes in plasma volume during posture change in healthy individuals (Fawcett & Wynn, 1960). However, several problems arise with the use of isotope-labeling techniques in the assessment of acute and continuous changes in plasma volume. The first problem with this technique is one of time. Once a known quantity of isotope-labeled RBC's and albumin have been injected into a seated subject, blood samples have to be obtained every ten to

fifteen minutes for up to two hours in order to obtain a "disappearance curve" for the measurement of baseline red cell mass and plasma volume. This procedure is then repeated two to three weeks later, depending on the half-life of the radioactive isotopes used, with the subject in a standing position. The second problem with this technique is the use of repeated injections of low-level radioactive substances into healthy subjects. Although it is low-level radiation, the repeated injections lead to prolonged internal exposure to the isotopes and possible additive effects of the radioactive dosage (Marx, 1979).

Densitometry techniques. In 1969 a new method was introduced--the mechanical oscillator technique (MOT)--for the high-precision measurement of homogenous fluids and gases (Kratky, Leopold, & Stabinger, 1969). More recently, this technique has been used to precisely measure blood (Hinghofer-Szalkay, 1986), plasma (Kenner & Hinghofer-Szalkay, 1984), and lymph volume (Leopold, Hinghofer-Szalkay, Kenner, & Holzer, 1978) without the inaccuracies and side effects of the hematocrit or isotope-labeling techniques. Unlike the measurement of hematocrit which depends on centrifuge separation of cells and plasma, samples measured in the MOT do not need to be centrifuged and thus eliminate the error of trapped plasma. The MOT also allows for the continuous measurement of blood and plasma density without having to wait for the mixing of isotope-labeled blood cells in the system or

multiple study sessions.

As mentioned earlier, increased red cell mass is characterized by increased RBC's with no change in plasma protein concentrations, while transvascular fluid shifts are characterized by increased plasma protein concentration. By measuring changes in both blood and plasma density, one can detect whether the mechanism for changes in plasma volume are due to transvascular fluid shifts or due to increased red cell mass. Thus, an increase in blood and plasma density would indicate that plasma volume is affected by transvascular fluid movement, while increased red cell mass would be characterized by an increase in blood density with no change in plasma density.

Since its inception, the MOT has been used extensively in the study of blood and plasma density changes after changes in posture using tilt table procedures (Hinghofer-Szalkay & Moser, 1986; Hinghofer-Szalkay & Greenleaf, 1987). Hinghofer-Szalkay and Moser (1986) performed a series of studies that examined the effects of tilt table posture changes on blood and plasma density. In one study, five male subjects were subjected to three tilt periods in which the subjects spent two 45-minute periods lying horizontal (periods 1 and 3) and one period tilted 70° heads up (period 2) with a transition period of 5-seconds from one position to the next. Results indicated that there was a significant increase in blood and plasma density ($p<0.05$) and a 13.4% decrease in plasma volume

during tilt changes from supine to 70° heads up. In a second study addressing the time course of plasma volume changes in six men, Hinghofer-Szalkay and Moser observed that during twelve 12-minute staged increases (-6° to 90°) and decreases, blood and plasma density increased almost linearly with increased angles of tilt, while the average decrease in plasma volume from supine to 90° heads up was 17.9%. In each of these studies erythrocyte density, which is an index of mean corpuscular volume, was also assessed. However, changes in tilt had no effect on erythrocyte volume, thus indicating that intracellular fluid shift did not occur. Similar decreases in plasma volume have been found during changes in posture from a seated position to standing while using the MOT to continuously measure blood density (Hinghofer-Szalkay & Greenleaf, 1987). Thus, research on posture-mediated changes in plasma volume have shown consistent and robust decreases in plasma volume during a change in posture from a seated position to a standing position, and that the mechanism for this change is an increase in lower extremity hydrostatic pressure.

OVERVIEW OF THE PROPOSED STUDY

Although research indicates that acute psychological stress can lead to a decrease in plasma volume and hemoconcentration of blood cells and large protein particles, the mechanism or mechanisms underlying this process has not

been well-defined. Research has indicated that there are several possible mechanisms for decreased plasma volume: 1) transvascular fluid shifts; 2) increased red cell mass; and 3) transcellular fluid shifts. The purpose of the present study was to examine each of these potential mechanisms by measuring changes in mean corpuscular volume, blood density, and plasma density, as well as the time course of plasma volume change during psychological stress. It was hypothesized that the stress-induced mechanism for decreased plasma volume is increased transvascular fluid shifts, characterized by a rapid increase in plasma density during stress and a relatively rapid return to baseline levels (< 30-minutes) during a recovery period. It was also hypothesized that increased blood pressure, as defined by increased mean arterial pressure, will be related to increases in blood and plasma density and to decreases in plasma volume in comparison to other potential mechanisms such as increased red cell mass or transcellular fluid influx. The present study also examined possible gender differences in plasma volume changes and mechanisms during acute psychological stress since gender differences have been found in the concentration of blood constituents that are susceptible to hemoconcentration during decreased plasma volume (e.g., lipoproteins).

SUMMARY OF HYPOTHESES

1. Psychological stress and postural change from a seated to standing position will cause acute contracted plasma volume by increased transvascular fluid shift due to increased mean arterial pressure.

2. Psychological stress and postural change will not cause increases in red cell mass or mean corpuscular volume.

3. Men, in comparison to women, will exhibit greater mean arterial pressure and acute contracted plasma volume responses to psychological stress and postural change.

4. The time course of acute contracted plasma volume will mirror changes in mean arterial pressure.

METHODS

Study Overview and Design

The present experiment was conducted as a 2 x 2 repeated measures design with gender (male/female) and group (stress/no stress) as between subjects' factors. The repeated measures factor was Task (baseline, mental arithmetic, and standing in the experimental group; baseline, Reading, and standing in the no stress group). The dependent measures in this study were

blood density (BD), plasma density (PD), plasma volume (PV), mean corpuscular volume (MCV), and mean arterial pressure (MAP) responses to the tasks. In addition, to determine the relationships between hemodynamic reactivity and various acute measures of contracted plasma volume, inter-relationships among blood density (BD), plasma density (PD), plasma volume (PV), mean corpuscular volume (MCV), and mean arterial pressure (MAP) were examined. The purpose of the no stress group was to assess possible changes in plasma volume due to vocalization-induced hemodynamic reactivity (see Reading), as well as, the possible effects of repeated blood drawing on changes in plasma volume.

Subjects

Forty subjects (20 men and 20 women) between the age of 18 and 45 years were recruited through bulletin board advertisements for a study examining "cognitive performance and hematology". Subjects were screened for any major health problem such as coronary disease or hypertension, or if any medication is regularly taken which may affect physiological responses (e.g., diuretics, antidiuretics, beta-blockers, birth control pills) (see Appendix A). Participants accepted into the study reported no medical conditions; no use of prescribed or illicit drugs or medications; no problems with alcohol; and no current or recent involvement in therapy with a psychologist or psychiatrist. Also, only subjects who were

non-smokers were included in the study due to the fact that smokers have lower plasma volume than non-smokers (Isager & Hagerup, 1971). In order to control for any short-term dietary or post-prandial effects on hematologic or hemodynamic variables (Lipsitz, Nyquist, Wei, & Rowe, 1983; Robertson et al., 1978), all subjects were asked to fast for 12 hours prior to the study and to abstain from drinking any caffeinated beverages on the morning of their scheduled appointment. Both male and female subjects were randomly assigned into 2 groups; stress and no stress.

Due to the fact that post-menopausal women have been reported to be more reactive to psychological stressors than pre-menopausal women (Polefrone & Manuck, 1988), a forty-five year age cut-off was chosen in order to ensure that most of the women recruited for the study will be pre-menopausal. Confirmation of each women's menopausal status was determined during the telephone screening.

As mentioned earlier, the results of studies assessing the effects of different menstrual cycle phases on cardiovascular (Polefrone & Manuck, 1988; Hastrup & Light, 1984; Carroll et al, 1984) and plasma volume changes (Gaebelein & Senay, 1982; Wells & Horvath, 1973) have not clearly demonstrated any single phase as affecting cardiovascular or plasma volume changes more that any other phase. However, although there appear to be few reliable menstrual cycle effects on cardiovascular and hematologic

reactivity, it is still important methodologically to establish consistent indexing of subject's menstrual phase and to study female subjects at comparable points in the menstrual cycle. Thus, the laboratory sessions for all female subjects in the present study were scheduled during the follicular phase of their menstrual cycle as defined as 1 to 11 days post menses.

Experimental Protocol

Each subject was screened by telephone to determine eligibility for participation. Once a subject was identified as being eligible to take part in the study, a description of the study was given and a suitable day for their laboratory session was scheduled. As mentioned above, to control for postprandial effects on plasma protein concentrations, each subject was instructed to fast for 12 hours prior to their scheduled appointment and to abstain from caffeinated beverages on the morning of the study.

Upon arrival to the laboratory, subjects were measured for height and weight. Subjects were then seated and informed consent obtained (see Appendix B). Lean body weight was then measured using bioelectrical impedance (RJL Systems, Inc.) in which electrical resistance was measured across electrodes that were placed on the right hand and foot. Lean body weight was then calculated using each subjects height, weight, and the electrical resistance of body composition. To ensure that all subjects fasted prior to their study session, each subject

filled out a questionnaire asking what and when they last ate (see Appendix C). An intravenous catheter (19-gauge butterfly needle) was then inserted into a vein at the antecubital fossa and flushed with 1 cc heparin (1000 units/ml) to keep the catheter patent. An inflatable cuff was then applied to the arm opposite the catheter and connected to an automated blood pressure monitor (Dinamap 864) for the measurement of heart rate (HR), systolic blood pressure (SBP), diastolic blood pressure (DBP), and mean arterial blood pressure (MAP) at the specified sampling time points. The blood pressure monitor automatically printed out SBP, DBP, HR, and MAP for each time point. Subjects were then instructed to relax and rest quietly during the 30-minute resting baseline period. Three blood pressure and heart rate readings were taken and recorded during the last 10 minutes of the resting baseline period in order to establish baseline hemodynamic levels. Three 4-ml blood samples were also taken during the last 10 minutes of the resting baseline period to establish baseline levels for plasma volume, blood density, plasma density, and mean corpuscular volume. One 10-minute psychological stressor or reading task and a 10-minute standing procedure were administered in random order after the 30-minute baseline period. Mental arithmetic (serial subtraction task with verbal harassment from an experimenter) was chosen since it produces consistent hemodynamic reactivity in both healthy men (Patterson et al, in press) and women (Girdler et al, 1990).

Passive reading of serial subtraction answers for the no stress group was chosen in order to control for the possible influence of vocalization on hemodynamic reactivity (Lynch, Thomas, Long, Manalow, Chichadonz, & Katcher, 1980). Standing was chosen as a physical task on the basis of its ability to produce decreased plasma volume without affecting red blood cell mass (Jensen, Glud, & Arnfred, 1984)(see Standing section). The standing procedure (subject went from a seated to standing position and remained standing for 10-minutes) was used as a comparison task with the psychological stressor in terms of examining the possibility for psychological stress-mediated changes in red blood cell mass. Subjects were told to relax again for the second resting period, lasting for 30-minutes, between the math or reading task and standing. At the end of each rest period and after the math or reading task and standing, state measures of affect were taken. At the end of the study, the catheter was removed, and the subjects were debriefed and paid $25 for their participation in the study.

Psychological and Physiological Stressors

The following tasks were administered after the initial resting baseline period. In order to control for task order effect, the task order was counterbalanced within each group.

Mental Arithmetic. Subjects in the stress group were given tape recorded instructions for the 10 minute mental arithmetic task. The tape recorded instructions for the task

were as follows:

"The following performance task is like the type that sometimes appear in math aptitude examinations. Specifically, we are interested in physiological changes that can occur while performing arithmetic equations in your head. Here are the instruction for the task, please listen carefully. Several times during the next ten minutes you will be asked to subtract from a four digit number by sevens or seventeens. Your job is to subtract from that four digit number as fast and accurately as you possibly can until I tell you to stop. For example, I may say, "Begin subtracting by sevens from the number 1169", you would say, "1169, 1162, 1155, 1148, etcetera, until you are told to stop. During this period we will be recording both the speed and accuracy of your performance. If you do not try just as hard as you can, we will not be able to gather accurate physiological information.

Now here is another important part of the instructions. Several times throughout the task you will be told to stop and begin subtracting by sevens or seventeens from a new four digit number. Your job again is to subtract by seven or seventeen from the new four digit number as fast as you can.

> Remember that for our measurements it is important that you keep still so that we can obtain accurate physiological information. If you have any questions ask them now. We will begin in a few moments. Remember to work as quickly and as accurately as you possibly can. The task will begin now. Begin subtracting by sevens from the number 1169."

Throughout the task, a recorded metronome sound was be played while the subject is periodically harassed by a second experimenter to speed up or be more accurate (Patterson et al, under review). Although there is much controversy over the effects of gender-relevant psychological stressors on cardiovascular reactivity (Saab, 1987), the serial subtraction task with harassment has been found to produce statistically similar cardiovascular reactivity in both men and women (Girdler et al, 1990).

Reading. Subjects were instructed to read a series of three and four digit numbers aloud from several sheets of paper at their own pace without any interruptions or harassment. The numbers that the subjects read aloud were the answers to the mental arithmetic serial subtraction task performed by subjects in the stress group (see Appendix D). The decision to use a vocalization task for the no stress group is based on the effects of vocalization on cardiovascular functioning (Lynch et al, 1980). Lynch et al.

demonstrated that non-stressful vocalization can lead to an increase in blood pressure. Since one of the main hypotheses of this experiment is that mean arterial pressure affects plasma volume, the vocalization task for the no stress group was used to examine the possible effects of vocalization-induced hemodynamic reactivity on changes in plasma volume and to rule out the possible vocalization effects on plasma volume in the stress group during the mental arithmetic task which requires vocalizing the answers to subtraction problems.

Standing. Subjects were asked to stand in place for 10 minutes at the end of the forty-minute seated rest period following the mental arithmetic task. The decision to use postural change was based on the well documented evidence that rising from a seated to standing position produces a significant change in plasma volume (Tan et al, 1973; Hinghofer-Szalkay & Moser, 1986) with no change in red blood cell mass (Fawcett & Wynn, 1960; Jensen et al, 1984). By using the postural change (standing) task, a comparison can be made between blood and plasma density measures (see Dependent Measures section) taken during the standing task and those taken during the psychological stress task in order to assess any psychological stress-mediated changes in red blood cell mass without having to use nuclear medicine techniques (e.g., red blood cell labeling with radioactive chromium-51 or technetium-99m). Since the first 15 seconds following postural change are accompanied by hypotension, the first

blood pressure reading and blood samples for the standing procedure were taken at 2 minutes into the posture change in order to avoid the possible confounding influence of the orthostatic or postural hypotension.

Measurement of Cardiovascular Reactivity

Heart rate (HR), systolic blood pressure (SBP), diastolic blood pressure (DBP), and mean arterial blood pressure (MAP) were measured and recorded three times during the last ten minutes of the baseline period, every two minutes during the math task and standing procedure, and every three minutes during the second rest period using a Dinamap (Model 846) automated blood pressure monitor. Mean arterial pressure was assessed because it is the pressure in the arteries averaged over time and depends on the mean volume of blood in the arterial system and the elastic properties of the arterial walls. MAP is thus deemed to be an important measure in this research since the main focus is on potential fluid shifts across the precapillary beds and into the interstitial spaces. The sampling time points for the hemodynamic measures coincided with the blood draws for the hematologic variables to allow for direct comparisons. The blood pressure cuff was attached to the arm opposite the indwelling catheter and the subjects were instructed to not move while the blood pressure cuff is inflating.

Measurement of Hematological Variables

Blood samples for hematocrit, hemoglobin, total plasma protein, mean corpuscular volume, blood density, and plasma density were obtained three times during the last ten minutes of the initial resting baseline period, every two minutes during the psychological stressor and standing task, and every 3 minutes during the second rest period. One 4 ml sample was taken at each of the sampling time points.

Plasma volume. Estimated plasma volume was arithmetically calculated from the baseline and stressor period hematocrit (Hct) and hemoglobin (Hb) values in a method described by Dill and Costill (1974). The formula used is as follows:

$$PV_A = (100 \text{ X } Hb_B/Hb_A) - [(100 \text{ X } Hb_B/Hb_A) \text{ X } Hct_A]$$

where Hct_A is the milliliters of red cells per milliliter of blood after mental arithmetic or standing, Hb_B is the hemoglobin concentration before and Hb_A is hemoglobin after each task. Hence, the percentage of change in plasma volume (%ΔPV) can be calculated as:

$$\%\Delta PV = 100 \ (PV_A - PV_B)/PV_B$$

where PV_B is equal to 100 - the baseline Hct (%).

Since changes in the actual size of red blood cells can

affect the packed cell volume of hematocrit (van Beaumont, 1972), hemoglobin is used in the plasma volume equation in order to control for possible changes in mean corpuscular volume. Although more direct measures of plasma volume are available (i.e., Evans blue dye, iodinated ^{125}I serum albumin), their limitations make them less than desirable in human studies assessing changes that occur over minutes or even hours (Greenleaf, & Hinghofer-Szalkay, 1985; Marx, 1979). As mentioned earlier, the primary limitations of the nuclear medicine techniques are the exposure of healthy subjects to radioactive substances and the amount of time that is required to assess plasma volume changes in each subject (2 weeks). Further support for the estimated plasma volume technique (Dill & Costill, 1974) comes from research that has shown this technique to been highly correlated (r=0.99) with the more direct nuclear techniques (Greenleaf, Convertino, & Mangseth, 1979).

Hematocrit levels and hemoglobin concentrations were determined using a Baker System 9000 cell counter (Serono-Baker), in which hematocrit levels were calculated from the red cell concentration and impedance determined mean corpuscular volume. Hemoglobin concentrations were determined by the cyanmethemoglobin method.

Mean corpuscular volume. Changes in mean corpuscular volume were measured by impedance detection with the Baker System 9000 cell counter (Serono-Baker).

Red blood cell mass. Blood and plasma density were used in conjunction with total plasma density to indirectly measure changes in red blood cell mass. Blood and plasma density were measured using a mechanical oscillator technique to detect mass density, which is defined as mass per unit volume. Densitometry measurements were made with the density-measuring apparatus (DMA) 55 and the DMA 602M units (Anton PAAR USA, Richmond, VA), in combination with ultrathermostats (Anton PAAR USA, Richmond, VA), which hold the operating temperature with 0.02-K precision. The DMA 602M contains a sample measuring cell that requires a 0.06 ml sample for mass density measurement. The DMA 602M was connected to the DMA 55 which contains a microprocessor unit that performs the actual density calculation and provides a visual display for data output. Total plasma protein was also measured to detect changes in plasma density using the Biuret method (Flack & Woollen, 1984).

Measurement of Affective State

At the end of each rest period and immediately following the mental arithmetic task and standing procedure, a measure of state affect was administered. State affect was used as a manipulation check for the psychological stressor to ensure that it was experienced as stressful. A paper and pencil questionnaire asking how happy, bored, anxious, satisfied, depressed, interested, angry, frustrated, restless, or

irritated the subject was feeling during each rest and task period was filled out by the subject at the end of each task (see Appendix E). Subjects were also asked to rate how challenging and difficult they found each task to be. Subjects drew a line through a 10 centimeter scale ranging from 0 cm ("not at all") to 10 cm ("extremely") to indicate how they were feeling during the rest periods and each task. Scales of this type have been found to be sensitive markers of mood state changes (Monk, 1989).

Power Analyses

The number of subjects for this study was determined based on data from previous studies of acute stress effects (Patterson et al, in press) and physical activity effects (Senay, 1972; 1973) on plasma volume responses. A power analysis was performed on the means and standard deviations of plasma volume during mental arithmetic stress to calculate an alpha level of 0.05 and a power level of 0.80 according to standard statistical procedures (Cohen, 1988). Since there are currently no studies that have assessed gender differences in plasma volume changes during psychological stress, a power analysis was performed using studies that have detected differences in lipid concentrations between men and women during stress (Stoney et al, 1988; Matthews et al, 1991). Using an alpha level of 0.05 and a power level of 0.80, 8 male and female subjects were required in each group for a total of

40 subjects (20 men and 20 women) in the study.

Statistical Analyses

Five 2 (gender) x 2 (group) between-subjects repeated-measures ANOVAs were used to examine the dependent repeated measures of BD, PD, PV, MCV, and MAP responses to psychological stress and posture change. These repeated measures ANOVAs were used to test the hypotheses that decreased plasma volume is caused by increased mean arterial pressure and transvascular fluid shift and not by increased red cell mass (Hypotheses 1 and 2). In order to test for gender differences in stress-induced plasma volume changes (Hypothesis 3), a 2 (gender) x 2 (group; stress/no stress) repeated-measures ANCOVA was conducted to control for weight difference between genders.

Subsequent correlational analyses using Pearson product-moment correlations were performed to determine the relationships among plasma volume changes and changes in blood pressure, blood density, plasma density, and mean corpuscular volume during mental arithmetic, reading, and standing.

In order to determine the time course of the plasma volume changes during psychological stress and recovery, Pearson product-moment correlations were performed to determine the relationships among plasma volume changes and changes in blood pressure across time. Repeated measures ANOVA was used to determine the length of time that it took

for plasma volume to return to normal during the recovery period (Hypothesis 4). The same repeated measures approach was also used to assess the time course for systolic, diastolic, and mean arterial pressure during psychological stress.

Human Subjects Protection

Forty subjects (20 men and 20 women), between the ages of 18-45, were recruited through bulletin board advertisements. Subjects were interviewed via telephone and were excluded for any major medical problems, use of medication that indicates a medical condition, or use of hematology altering medications (e.g., aspirin, aspirin containing medications). Subjects were informed about the study during the telephone interview and again at the beginning of the laboratory session, after which informed consent was obtained. Subjects were thoroughly informed about their right to discontinue participation at any time during the study. Possible discomfort to the subject was the insertion of the butterfly needle and inflation of the blood pressure cuff. No other discomfort was expected.

RESULTS

Sample Characteristics.

The sample consisted of 40 men and women, who were assessed through telephone screening as physically and psychologically healthy and ranged in age from 18 to 45. The

sample was relatively homogeneous in age (mean = 30.05 years, SD = 7.36 years, range 18-45), height (mean = 67.2", SD = 7.1"), weight (mean = 153 lbs, SD = 48.43 lbs), lean body weight (mean = 123.47 lbs, SD = 35.34 lbs), and race (72% white, 20% African-American, and 7.5% Asia-American). Almost all subjects reported no family history of hypertension (95%). There were no differences between the stress and no stress groups for any of the demographic or physical characteristic variables (see Table 1).

Differences in lean body weight between men and women was assessed because of the possible confounding effect of adipose tissue on plasma volume (Gregerson & Rawson, 1959). Lean body weight (normal body weight minus adipose tissue weight) was calculated from height, weight, and the electrical resistance of body composition. Analysis of variance comparing men and women on height, weight, and lean body weight revealed significant differences for height, $F(1,39) = 4.65$, $p < .05$, weight, $F(1,39) = 10.87$, $p < .001$, and lean body weight $F(1,39) = 11.34$, $p < .001$, with men being taller (mean = 70.2" vs. 64.2"), heavier in total body weight (mean = 173.9 lbs vs. 137.45 lbs) and lean body weight (mean = 141.7 lbs vs. 99.25 lbs) than women (see Table 1).

Effectiveness of the Stress Manipulation

Subjects were randomly assigned to perform the mental

arithmetic (stress) task or a reading (no stress) task in which numbers were read aloud slowly in order to control for effects of vocalization on hemodynamic and hematologic variables. At the end of the baseline period and immediately following the mental arithmetic task and standing procedure, a visual analog scale was completed that included 10 adjectives (happy, bored, anxious, satisfied, depressed, interested, angry, frustrated, restless, and irritated) and 2 questions ("how challenging was the task?" and "how difficult was the task?"). Subjects were asked to rate (from "not at all" to "extremely") each adjective and question following each task. Multivariate analysis of variance (MANOVA) was used to compare men and women on the subjective ratings of mood states.

A 2 x 2 MANOVA using the between subjects factors of group and gender on all of the mood ratings was conducted. Results revealed significant multivariate main effects for group $F(1,70) = 13.78$, $p < .001$ and mood measure $F(11,374) = 5.93$, $p < .001$, and a significant group x mood measure interaction $F(11,374) = 9.70$, $p < .001$. No significant gender main effects or interactions were demonstrated. Univariate ANOVAs revealed that subjects in the stress group rated themselves as being significantly more anxious $F(1,18) = 19.17$, $p < .001$, angry $F(1,18) = 16.46$, $p < .001$, frustrated $F(1,18) = 39.21$, $p < .001$, and irritated $F(1,18) = 25.7$, $p < .001$ during the mental arithmetic task compared to the no

stress group during the reading task (see Figure 2). Stress group subjects also rated themselves as being significantly more challenged $F(1,18) = 145.52$, $p < .001$ and their task (mental arithmetic) as significantly difficult $F(1,18) = 194$, $p < .001$ compared to the no stress group (see Figure 3). Univariate analyses also revealed that women in the stress group reported the mental arithmetic task as being significantly more difficult than did men. The no stress group, on the other hand, rated themselves significantly more bored during the reading task $F(1,18) = 3.88$, $p < .01$ (see Figure 4). No significant main effects were found for any of the self-report items during the standing task. Thus, the self-report data indicate that the experimental manipulation was effective in producing increased emotional arousal in both men and women in the stress group and that there were no significant gender differences in self-report arousal.

There were 4 specific hypotheses tested in this study. The results of the data analyses used to test these hypotheses are presented below.

Stress Induced Blood Pressure Changes

During the last 10 minutes of the baseline period, hemodynamic (SBP, DBP, HR, and MAP) and hematological (MCV, TPP, BD, and PD) measures were taken every three minutes and averaged. Analysis of variance (ANOVA) procedures were used

to compare men and women on baseline SBP, DBP, HR, and MAP. Considering the averaged levels for each of the hemodynamic variables at baseline, SBP among men was significantly higher than among women $F(1,39) = 4.11$, $p < .05$. There were no significant gender differences for baseline DBP, HR, or MAP (see Table 2).

To assess the effects of psychological stress and postural change on blood pressure, a series of MANOVAs were conducted. Since the standing task, which is known to increase blood pressure, was performed by both groups, two 2 x 2 MANOVAs were conducted, one to assess the effects of math versus the reading task, and one to assess the effect of standing on blood pressure. Both 2 x 2 MANOVAs were conducted with group and gender as between-subjects factors. The dependent variables for the first MANOVA were mean SBP, DBP, and MAP during baseline, math, and reading. Results revealed a significant multivariate main effect for group $F(1,35) = 56.78$, $p < .001$ and a significant group x task interaction $F(3,105) = 35.56$, $p < .001$. No other multivariate main effects or interactions were demonstrated. Univariate analyses indicated that the stress group had significant increases in SBP $F(3,108) = 25.84$, $p < .001$, DBP $F(3,108) = 30.62$, $p < .001$, and MAP $F(3,114) = 28.9$, $p < .001$ during mental arithmetic (see Figures 5-7). No significant blood pressure changes occurred during reading in the no stress group.

The dependent variables for the second MANOVA were mean

SBP, DBP, and MAP during baseline and standing task. A significant multivariate main effect for task was indicated $F(2,70) = 25.72$, $p < .001$. No other multivariate main effects or interactions were demonstrated. Univariate analyses of the stress and no stress groups separately revealed significant increases in SBP $F(2,70)$, $= 15.57$, $p < .001$, DBP $F(2,70) = 47.52$, $p < .001$, and MAP $F(2,70) = 42.20$, $p < .001$ in the stress group (see Figures 5-7). Significant increases in SBP $F(2,70) = 20.12$ $p < .001$, DBP $F(2,70) = 34.45$, $p < .001$, and MAP $F(2,70) = 38.67$, $p < .001$ were also found in the no stress group (see Figures 4-6). Newman-Keuls post hoc comparisons indicated that there were no significant differences between groups for PV, SBP, DBP, or MAP during the standing task.

Stress Induced Hematologic Changes

Plasma volume. Baseline hematological (BD, PD, TPP, and MCV) measures were taken every three minutes during the last 10 minutes of the 30 minute baseline period and then averaged. An ANOVA was used to compare men and women on baseline PV using the following formula to determine baseline PV:

PV = 100 - baseline hematocrit value

PV was found to be significantly higher in men than in women $F(1,39) = 15.98$, $p < .001$. Similar gender comparisons of BD, PD, TPP, and MCV revealed no significant gender differences

for baseline levels (see Table 3).

Since both groups performed the standing task which is known to produce decreases in plasma volume, the same analysis strategy used for the blood pressure analyses was employed to assess the effects of psychological stress and postural change on plasma volume. Two 2 x 2 ANOVAs were conducted, one to assess math and reading task effects and one to assess the standing task effects on plasma volume. Both 2 x 2 ANOVAs were conducted with group and gender as between-subjects factors and trials as a within-subjects factor. The dependent variable for the first ANOVA was mean PV during baseline, math, and reading. Results revealed a significant main effect for group $F(1,36) = 327.86$, $p < .001$ and a significant group x task interaction $F(3,108) = 284.34$, $p < .001$. No other main effects or interactions were demonstrated. When the groups were analyzed separately, no significant main effects or interaction were demonstrated for the no stress group. For the stress group, a significant decrease in PV $F(3,108) = 639.09$ $p < .001$ was indicated (see Figure 8).

The dependent variable for the second ANOVA was mean PV during baseline and standing task. The results indicated a significant main effect for group $F(1,36) = 439.09$ $p < .001$. No other main effects or interactions were demonstrated. Newman-Keuls post hoc comparisons indicated that there were no significant differences between groups for PV during the standing task.

Since main effects for task on plasma volume were found for the stress group in both analyses, differential task effects on PV during baseline math and standing were assessed using a repeated measures ANOVA and Newman-Keuls post hoc comparisons. Results, again revealed a significant main effect for task on PV $F(3,54) = 326.24$, $p < .001$. Newman-Keuls post hoc comparisons between stress and standing indicated that in the stress group, PV decreased significantly more during the standing task than during mental arithmetic ($p < .05$).

Because it is possible that gender differences in plasma volume are a result of differences in the amount of adipose tissue found in men and women, lean body weight (total body weight minus adipose tissue weight) was used as a covariate to control for body composition effects on plasma volume. An analysis of covariance was conducted using PV changes scores (task - baseline level) during the math, reading, and standing tasks. Similar to the gender difference analyses mentioned above, gender differences were not found for PV after controlling for lean body weight.

Red cell mass. Stress and postural change were not expected to cause increases in red cell mass or mean corpuscular volume (Hypothesis 3). In order to test this hypothesis, two sets of analyses were performed. The first set of analyses examined relationships between changes in

plasma volume (PV) and increases in red cell mass. This was tested by measuring changes in blood density (BD) and plasma density (PD). An increase in red cell mass would be indicated by a significant increase in blood density, with no change in plasma density. A true decrease in plasma volume would be indicated by a significant increase in both blood density and plasma density. Moreover, these measures (blood density and plasma density) should be correlated with one another. The change in total plasma protein (TPP) was also assessed since decreases in plasma volume would produce an increase in TPP, whereas increased red cell mass would have little effect on TPP. Using the same analysis strategy as described above, two 2 x 2 MANOVAs were conducted, one to assess math and reading task effects and one to assess the standing task effects on BD, PD, and TPP. Both 2 x 2 MANOVAs were conducted with group and gender as between-subjects factors. The dependent variables for the first MANOVA were mean BD, PD, and TPP during baseline, math, or reading. Results revealed a significant multivariate main effect for group $F(1,35) = 15.57$, $p < .001$. Results also indicated a significant group x task interactions $F(2,78) = 7.52$, $p < .001$. There were no gender main effects or interactions demonstrated. Univariate analyses revealed that the stress group had a significant increase in BD $F(3,108) = 92.69$, $p < .001$, PD $F(3,108) = 49.88$, $p < .001$, and TPP $F(3,108) = 117.06$, $p < .001$ during mental arithmetic (see Figures 9-11).

The dependent variables for the second MANOVA were mean BD, PD, and TPP during baseline and standing task. A significant multivariate main effects for task was found $F(2,70) = 66.18$, $p < .001$. No other multivariate main effects or interactions were found for BD, PD, or TPP during the standing task. Univariate analyses of each group separately revealed significant increases in BD $F(2,70) = 168.98$, $p < .001$, PD $F(2,70) = 89.56$, $p < .001$, and TPP $F(2,70) = 124.20$, $p < .001$ in the stress group and significant increases in BD $F(2,70) = 201.45$ $p < .001$, PD $F(2,70) = 102.87$, $p < .001$, and TPP $F(2,70) = 113.34$, $p < .001$ in the no stress group (see Figures 9-11). Newman-Keuls post hoc comparisons indicated that there were no significant differences between the stress and no stress group for BD, PD, or TPP during the standing task.

Mean corpuscular volume. To test the hypothesis that changes in plasma volume were not due to stress-induced increases in mean corpuscular volume (MCV; i.e., influx of extracellular fluid into red blood cells) (Hypothesis 3), changes in MCV were assessed during math, reading, and standing. The analysis strategy of conducting separate analyses for the math and read tasks and the standing task was also used for analyzing MCV changes. Two 2 x 2 ANOVAs were used to assess task effects on MCV and were conducted with group and gender as between-subjects factors and the within-

subjects variable being MCV during baseline, math, or reading. Results indicated that there were no significant main effects or interactions demonstrated during mental arithmetic or reading (see Figure 12). The dependent variable for the second ANOVA was mean MCV during baseline and standing task. Results indicated that there were no significant main effects or interactions demonstrated during standing (see Figure 12).

Relationship Between Blood Pressure and Plasma Volume

To assess the relationship between changes in plasma volume and changes in blood pressure, Pearson product-moment correlational analysis was used (Hypothesis 2). Correlations between changes in calculated plasma volume and changes in blood pressure were computed using change scores for PV, SBP, DBP, and MAP (task - baseline levels) during mental arithmetic and standing. Correlation analyses were also performed on changes in these variables during the reading task. Results revealed a significant negative correlation between changes in PV and SBP during mental arithmetic ($r = -.51$, $p < .02$). A significant negative correlation was also found between PV and MAP changes during mental arithmetic ($r = -.47$, $p < .04$). Surprisingly, reliable correlations were found between PV and SBP, DBP, or MAP during the reading or standing task (see Table 4).

The same strategy used above for assessing the relationship between calculated plasma volume and blood

pressure was used to assess the relationship between changes in blood pressure and changes in blood and plasma density during mental arithmetic, reading, and standing. Results indicated a strong correlation between BD and SBP ($r = .48$, $p < .03$) and MAP ($r = .45$, $p < .05$) during mental arithmetic (see Table 5). A strong correlation was also found between changes in PD and SBP ($r = .54$, $p < .02$) and MAP ($r = .49$, $p < .03$) during mental arithmetic (see Table 6).

Pearson product-moment correlational analysis was also used to assess relationships among change scores for BD, PD, and TPP during mental arithmetic (math - baseline levels), standing (standing - baseline levels), and during the reading task (reading - baseline levels). Results indicated a significant correlation between changes in BD and PD during mental arithmetic ($r = .64$, $p < .001$) and standing ($r = .69$, $p < .001$). A significant correlation was also found between PD and TPP mental arithmetic ($r = .44$, $p < .05$) and standing ($r = .62$, $p < .001$). No reliable correlations were found between BD and PD, or TPP during the reading task (see Table 7).

Since increases in both blood density and plasma density are direct evidence for decreases in plasma volume, correlations were used to assess the relationship between changes in calculated PV and changes in BD and PV during mental arithmetic, reading, and standing. Results indicated a reliable inverse relationship between PV and BD during math ($r = -.47$, $p < .04$) and standing ($r = -.51$, $p < .001$) and

between PV and PD during math ($r = -.51$, $p < .02$) and standing ($r = -.53$, $p < .001$) (see Table 4). The strong association between these two indices of plasma volume indicate a reliable alteration in plasma during psychological stress and postural change.

Time Course of Plasma Volume and Blood Pressure Changes

The time course of acute contracted plasma volume changes were expected to mirror changes in mean arterial pressure during psychological stress (Hypothesis 4). This hypothesis was tested in two ways. First, plasma volume and blood pressure levels were correlated across baseline, math task, and recovery periods using a Pearson product-moment correlation of all the sampling time points during baseline (3 time points), task (5 time points), and recovery (10 time points) for each variable. Results of the correlation revealed a strong correlation between PV and SBP ($r = .55$, $p < .0001$), DBP ($r = .61$, $p < .0001$), and MAP ($r = .65$, $p < .0001$).

Since blood pressure and plasma volume levels during the mental arithmetic task appeared to peak at different times (2-4 minutes into the task for SBP, DBP, and MAP; 8 minutes into the task for PV), a lag correlation procedure was used to determine differences in the association between plasma volume and blood pressure due to a lag in peak times. In a lag correlation, the first time point is removed from the variable

that has the longest latency in peak time and the last time point is removed from the variable with the shortest latency in peak time. The removal of one time point from each variable is thus called lag 1, the removal of 2 time points is called lag 2. Two separate Pearson product-moment correlations were computed using lag 1 and lag 2. Results of the lag 1 correlation revealed a somewhat lower correlation between PV and SBP ($r = .43$, $p < .0001$), DBP ($r = .32$, $p < .0001$), and MAP ($r = .47$, $p < .0001$). Results of the lag 2 correlation revealed the lowest significant correlation between PV and SBP ($r = .32$, $p < .001$), DBP ($r = .20$, $p < .001$), and MAP ($r = .34$, $p < .001$). The result of these three correlations thus indicate that the strongest relationship between blood pressure with plasma volume occurred during the true sampling periods of both variables.

The second analysis of the blood pressure-plasma volume time course required using repeated measures ANOVAs to assess similarities in the recovery of both plasma volume and blood pressure following termination of the stressor. The repeated measures terms were PV and MAP during baseline, mental arithmetic, and each sampling point during the recovery period (every 3 minutes). Results indicated that there was still a marginally significant decrease in PV by the third sampling period of the recovery period (9 minutes post stress) $F(1,19) = 3.92$, $p < .07$. By the fourth sampling period of the recovery period changes in PV were no longer significant ($p >$

.32). The results for MAP were similar in that MAP remained significantly elevated through nine minutes of the recovery period $F(1,19) = 5.25$, $p < .03$ and was no longer significantly elevated at 12 minutes post stress ($p > .17$)(see Figure 13). Thus, the strongest association found between plasma volume and blood pressure occurred in the unadjusted correlational analyses followed by the lag 1 and lag 2 associations, therefore indicating that a strong reliable relationship exists between plasma volume and blood pressure regardless of the lag in peak times during stress.

DISCUSSION

The principal findings of this study were that stress-induced decreases in plasma volume in both men and women were reliably associated with increases in blood pressure and that the time course of both plasma volume and blood pressure changes coincided during the stress task and recovery period.

The hypothesis that men, in comparison to women, would exhibit greater blood pressure and plasma volume responses to psychological stress and postural change was not supported by the present study. Although men exhibited significantly higher SBP baselines and lower PV baselines than women, no gender differences in blood pressure changes, plasma volume changes, or any of the other hematological variables were found during psychological stress or posture change. Since plasma volume levels are influenced by individual differences

in adipose tissue, gender differences in plasma volume during stress was also assessed by covarying for lean body weight. Even after controlling for adipose tissue, no gender differences in plasma volume were demonstrated.

Support was obtained for the hypothesis that psychological stress-induced acute changes in plasma volume result from a mechanism involving transvascular fluid shifts produced by acute increases in mean arterial pressure. However, there was little support for the hypothesis that postural-induced changes in plasma volume resulted from increased blood pressure. Specifically, subjects in the stress group exhibited significant increases in SBP, DBP, MAP, and a significant decrease in PV during the mental arithmetic and standing task. Surprisingly, only the changes in systolic blood pressure, mean arterial pressure, and plasma volume that occurred during psychological stress were correlated and not the changes that occurred during standing. One possible explanation for lack of a relationship between blood pressure and plasma volume during the standing task may be that the hemostatic pressure of venous pooling of blood in the legs following postural change (seated to standing) plays a more prominent role as a mechanism for posture-induced plasma volume change than does increased hemodynamic blood pressure alone. This possibility is further supported by the fact that while blood pressure increase peaked 2 minutes into the standing task and leveled off for the remainder of the task,

plasma volume continued to decrease throughout the entire task with peak decrease occurring at the end of the task.

Psychological stress and postural change did not cause increases in red cell mass or mean corpuscular volume. This conclusion was supported by two separate procedures in the present research. First, mass densitometry techniques were used to directly assess whether changes in plasma volume were due to a shift of fluid from the vascular compartment to the interstitial spaces or if the apparent change in plasma volume was actually due to an increase in the red cell mass. The benefit of using this technique is that plasma volume changes can be detected without the use of potentially harmful nuclear medicine techniques such as radioactive labelling of blood cells and serum albumin. By assessing changes in blood and plasma density, the former plasma volume change would reveal an increase in both blood and plasma density, while the latter mechanism would reflect only an increase in blood density. By contrast, psychological stress and posture change did not increase red cell mass. This was identified by the fact that <u>both</u> blood density and plasma density increased significantly during stress and standing, rather than an increase in only blood density. This conclusion was further supported by a strong relationship between changes in blood and plasma density changes during math and standing as well as the reliable relationship between changes in blood and plasma density and changes in blood pressure.

The second procedure assessed possible changes in mean corpuscular volume as a mechanism for changes in plasma volume during stress. The lack of an effect of stress or posture on mean corpuscular volume was supported by data indicating that MCV did not change significantly during mental arithmetic or standing. Although increased mean corpuscular volume was ruled out as a mechanism for decreased plasma volume, it should be pointed out that mean corpuscular volume should have decreased due to the increase in osmotic pressure from the increased total plasma protein levels. In this case red blood cells should have shrunk in size somewhat during psychological stress rather than mean corpuscular volume remaining unchanged. One possible explanation for the lack of a decrease in mean corpuscular volume may be that between the time that each blood sample was taken and the time in which mean corpuscular volume was actually analyzed on the Coulter counter fluid red blood cells could have reabsorbed fluid in the plasma and thus bringing the cells back to their normal size. This is especially plausible since the blood samples sat at room temperature for as long as 1 hour before being assayed.

Results of the present study also revealed that the time course of acute plasma volume contraction mirrors similar changes in mean arterial pressure. In this regard, plasma volume and blood pressure were found to be highly correlated and changes in both plasma volume and blood pressure following

the mental arithmetic task were found to return to baseline within 12 minutes following the termination of the task.

Comparison of the Present Results to Other Studies

Relatively few studies have examined the effects of psychological stress on plasma volume or have identified possible mechanisms for stress-induced changes. Muldoon et al (1992) reported that psychological stress produced by a 20-minute computerized Stroop and mental arithmetic task, as well as posture change from a seated to standing position, produced significant decreases in plasma volume in 26 healthy men. These results are similar to that of the present study in that stress and posture produced significant plasma volume changes. Unlike the present study in which plasma volume returned to baseline 12 minutes after the stressor termination, Muldoon reported that plasma volume remained significantly lower than baseline levels throughout the recovery period and suggested that the mechanism for the long term reduction in plasma volume following stress was either increased red blood cell counts due to splenic contraction or increased renal function and diuresis. Although renal function was not assessed in the present study, the data from the time course analysis in present study do not support increased renal function as a possible mechanism for stress-induced decreases in plasma volume due to the rapidity of the plasma volume change during

stress and subsequent return to baseline following stressor termination which is consistent with other studies finding similar rapid plasma volume return following stressor termination (Jern et al, 1991; Patterson et al, in press).

In another study conducted in our laboratory, psychological stress also produced acute decreases in plasma volume (Patterson et al, in press). However, unlike findings of Muldoon et al and more consistent with the present research, the Patterson et al (in press) study found that plasma volume returned to baseline within 30 minutes of termination of the stressor. This suggested that the mechanism for plasma volume changes would have to be one that facilitates rapid fluid changes such as increased hydrostatic pressure due to acute increases in blood pressure.

Two other studies have reported rapid changes in hematocrit values during psychological stress. Jern et al (1991) reported a significant increase in hematocrit value and hemoglobin levels during a 10 minute mental arithmetic stressor after which hematocrit and hemoglobin returned to baseline levels within 10 minutes post-stress. In another study that assessed changes in hematocrit values during mental arithmetic stress (Kitahara, Imataka, & Nakaoka, 1988), a significant correlation between changes in systolic blood pressure and hematocrit during stress was found. This hemodynamic mechanism is further supported by data from our laboratory demonstrating a significant negative association

between calculated plasma volume and changes in mean arterial pressure during stress (r=-0.67 and r=-0.49, respectively)(Patterson et al, under review). The relationship between plasma volume and blood pressure in the Patterson et al studies and in the present research is consistent and strongly supports a hemodynamic mechanism as a cause for the stress-induced changes in plasma volume.

However, it should be pointed out that in all of the above mentioned studies that observed plasma volume changes during psychological stress used mental arithmetic as the stress task which is known to be a potent stressor for eliciting strong cardiovascular responses. Future research is needed to assess the effects of various types of stressors on plasma volume and the plasma-blood pressure relationship.

Hemodynamic Mechanism for Plasma Volume Change During Stress

The present study suggests that the primary mechanism for stress-induced decreases in plasma volume is increased blood pressure; potential mechanisms such as increased red cell mass or increased mean corpuscular volume were ruled out. This relationship between blood pressure and plasma volume was determined in two ways. First, blood pressure changes were found to be reliably associated with changes in calculated plasma volume during mental arithmetic. More importantly, significant correlations were also found between changes in

blood pressure and changes in blood density, plasma density, and total plasma protein. These correlations lend strong support to the proposed hemodynamic mechanism for plasma volume change due to the direct nature of their measurement. The use of mass densitometry techniques in the present study is key to being able to directly assess rapid plasma volume changes over time.

Additional supporting evidence for a hemodynamic mechanism for plasma volume change was the similarity in the time course of both plasma volume and blood pressure changes during and after psychological stress. Although blood pressure and plasma volume peaked at different times during the stress period (2-4 minutes and 6-8 minutes respectively), a strong relationship was found between both variables during baseline, stress, and recovery. More striking is that the return of both blood pressure and plasma volume to baseline during the recovery period occurred at the same time and that both variables returned to baseline levels 12 minutes after the math task was finished.

Plasma Volume Changes in Men vs. Women

As mentioned previously, prior to the present research, no investigations had been conducted to address the possibility that gender differences exist in plasma volume changes during stress. Only studies on the effects of physical exercise and heat exposure have examined gender

differences in plasma volume changes. Studies by Sawka et al (1983, 1984) did not detect any gender differences in intravascular plasma volume responses during prolonged heat exposure or strenuous treadmill running with men and women matched for fitness level. Although the present research did not assess physical exercise effects on plasma volume in men and women, the results were similar in that no gender differences found in plasma volume changes during psychological stress. One possible explanation for the lack of a gender difference in plasma volume during stress in the present study is that the type of stressor used (mental arithmetic) and the method of task administration (intermittent harassment) produced similar cardiovascular responses in both men and women and thus the hemodynamic mechanism for the plasma volume changes was equally effective for both men and women. The similar cardiovascular responses for men and women found in the present study are consistent with other studies using the same stressor (mental arithmetic). Girdler et al. (1990) found no gender differences in blood pressure responses during a serial subtraction task as well as during a computerized math task. Similarly, Jorgensen and Houston (1981) failed to find gender differences in hemodynamic reactivity during mental arithmetic. However, the type of task used may not completely explain the similarity in blood pressure and plasma volume responses in men and women since several other studies

that employed the mental arithmetic task found significant differences between men and women (Matthews et al, 1991; Matthews & Stoney, 1988; Stoney et al, 1988). An alternative explanation could be that not only the stressor used in the present study affected the gender outcome for plasma volume and blood pressure responses, but that the particular phase of the menstrual cycle used in the study could have caused increased cardiovascular responding in women. This possibility is supported by research conducted by Polefrone and Manuck (1988) in which women in their follicular phase had greater blood pressure increases during mental arithmetic and concept formation tasks than women in their luteal phase. However, regardless of the fact that the hypothesis of gender differences during stress was not supported by the present study, the lack of a gender difference in the present research is of importance in that plasma volume changes need to be assessed in both men and women in psychophysiological stress studies where hemoconcentration effects may alter the levels of other biochemical substances.

CONCLUDING REMARKS

Identification of the mechanism for stress-induced plasma volume change has both methodological and clinical implications for future psychophysiological research. Methodologically, the present study has several potentially

wide-ranging implications. The phenomenon of stress-induced decreases in plasma volume suggests that studies examining stress-mediated changes in large biochemical substances found in the blood (e.g., lipids, proteins) need to take into account the possibility of systemic fluid shifts and to control for possible hemoconcentration effects. As indicated earlier, stress-induced changes in plasma volume have been shown to account for the changes found in cholesterol during acute laboratory stress (Patterson et al, in press; Muldoon et al, 1992). However, hemoconcentration effects may go well beyond this. For example, it is possible that research assessing acute changes in substances associated with the clotting process (e.g., fibrinogen, platelet proteins) or biochemical substances associated with immune function (e.g., interlukin 1, interlukin 2, interferon) during psychological stressors that produce substantial increases in blood pressure may need to take into account the possible effects of changes in plasma volume, since these biochemical substances do not passively pass through the vessel wall with the efflux of fluid. However, relatively few studies have addressed the possibility that stress-mediated plasma volume changes may influence the levels of these substances.

There are several implications of the present research that are of clinical relevance. It is possible that in patients with coronary artery disease, the effects of acute stress on plasma volume might contribute to myocardial supply

(e.g., coronary thrombosis, vasoconstriction, decreased microcirculatory flow) and demand (e.g., increased work load of pumping high viscosity blood). For example, an acute decrease in plasma volume leads to increased blood viscosity which has been found to be associated with several cardiovascular diseases (Chien, 1977; Dintenfass, 1977). Future research needs to address the possible relationship between acute stress-induced changes in plasma volume and coronary artery disease as a predisposing factor for atherosclerosis and as a possible mechanisms for acute cardiovascular events.

It is also possible that stress-mediated decreases in plasma volume may be a physiological link between the association of psychological stress and hypertension. Research has shown that patients with essential hypertension also have lower than normal plasma volume levels and increased blood viscosity which, in turn, places an added strain on the heart (Chien, 1977; Dintenfass, 1977). It is possible that in hypertensive patients, stress-induced decreases in plasma volume only compound the strain placed on the heart and thus potentially hasten the cardiovascular damage produced by elevated blood pressure. Again, future research is needed in this area in order to assess any possible connection between stress-induced plasma volume changes and hypertension.

SUMMARY

In sum, the present study found that psychological stress caused an acute decrease in plasma volume and that the decrease in plasma volume was negatively related to increased blood pressure in both men and women. The present study also found that postural change from a seated to standing position produced a significant decrease in plasma volume, but that the change in plasma volume during postural is not related to blood pressure changes. This study was also able to rule out increased red cell mass and increased mean corpuscular volume as potential mechanisms for stress-induced plasma volume changes since neither variable was affected by psychological stress. The present study however, failed to support the hypothesis that men, in comparison to women, would exhibit greater blood pressure and plasma volume responses to psychological stress and postural change since men and women exhibited similar responses in both variables during stress. Finally, the present study was able to demonstrate that the time course of acute contracted plasma volume mirrored the time course of changes in blood pressure during baseline, mental arithmetic, and recovery periods, thus further supporting a transvascular fluid shift mechanism for stress-induced changes in plasma volume.

TABLE 1

Comparison of Male and Female Subjects on Demographic and Physical Characteristics

Variable		Male (n=20)		Female (n=20)		Significance
AGE		29.85±7.4 yrs.		30.25±7.2 yrs.		ns
HEIGHT		70.20±2.9 in.		64.20±2.6 in.		$p < .05$
WEIGHT		173.90±28 lbs.		137.45±17 lbs.		$p < .001$
LEAN BODY WEIGHT		141.70±16 lbs.		99.25±9 lbs.		$p < .001$
		%	n	%	n	
RACE	White	37.5	15	35.0	14	
	Black	10.0	4	10.0	4	ns
	Other	2.5	1	5.0	2	
FAMILY HISTORY OF HYPERTENSION		2.5	1	2.5	1	ns

Note: Age, height, weight, and lean body weight are reported as mean ± S.D.

TABLE 2

Comparison of Male and Female Subjects on Baseline Hemodynamic Variables (mean±SD)

Variable	Male (n=20)	Female (n=20)	Significance
SBP (mmHg)	115.63±11	107.53±11	$p < .05$
DBP (mmHg)	67.61±9	66.58±9	ns
MAP (mmHg)	84.18±8	81.48±10	ns
HR	57.90±9	61.63±11	ns

Abbreviations: SBP = Systolic Blood Pressure, DBP = Diastolic Blood Pressure, MAP = Mean Arterial Pressure.

TABLE 3

Comparison of Male and Female Subjects on Baseline Hematologic Variables (mean±SD)

Variable	Male (n=20)	Female (n=20)	Significance
PV (%)	60.50±2.3	67.50±2.7	p <.0001
MCV (μ^3)	87.11±2.5	84.23±6.8	ns
TPP (g/dl)	6.23±0.4	6.17±0.4	ns
BD (g/cm^3)	1.0479±0.00	1.0458±0.00	ns
PD (g/cm^3)	1.0198±0.00	1.0196±0.00	ns

Abbreviations: MCV = Mean Corpuscular Volume, TPP = Total Plasma Protein, BD = Blood Density, PD = Plasma Density.

Table 4

Pearson Correlations Between Changes in Plasma Volume to Changes in Hemodynamic and Hematologic Variables During Mental Arithmetic, Reading, and Standing

Variable	Mental Arithmetic n=20	Reading n=20	Standing n=40
SBP (mmHg)	-.51 p <.02	-.05	.14
DBP (mmHg)	-.33	-.03	-.19
MAP (mmHg)	-.47 p <.04	-.08	-.11
MCV (μ^3)	.05	-.12	.03
TPP (g/dl)	-.46 p <.04	-.27	-.52 p <.001
BD (g/cm^3)	-.47 p <.04	.13	-.51 p <.001
PD (g/cm^3)	-.51 p <.02	-.03	-.53 p <.001

Abbreviations: SBP = Systolic Blood Pressure, DBP = Diastolic Blood Pressure, MAP = Mean Arterial Pressure, MCV = Mean Corpuscular Volume, TPP = Total Plasma Protein, BD = Blood Density, PD = Plasma Density.

Table 5

Pearson Correlations Between Changes in Blood Density to Changes in Hemodynamic Variables During Mental Arithmetic, Reading, and Standing

Variable	Mental Arithmetic (n=20)	Reading (n=20)	Standing (n=40)
SBP (mmHg)	.48 p <.03	-.13	.15
DBP (mmHg)	.28	.09	.16
MAP (mmHg)	.45 p <.05	-.12	.21

Abbreviations: SBP = Systolic Blood Pressure, DBP = Diastolic Blood Pressure, MAP = Mean Arterial Pressure.

Table 6

Pearson Correlations Between Changes in Plasma Density to Changes in Blood Pressure During Mental Arithmetic, Reading, and Standing

Variable	Mental Arithmetic (n=20)	Reading (n=20)	Standing (n=40)
SBP (mmHg)	.54 p <.02	-.01	.03
DBP (mmHg)	.30	.04	.23
MAP (mmHg)	.49 p <.03	-.04	.18

Abbreviations: SBP = Systolic Blood Pressure, DBP = Diastolic Blood Pressure, MAP = Mean Arterial Pressure.

Table 7

Pearson Correlations Between Changes in Plasma Density and Changes in Blood Density and in Total Plasma Proteins During Mental Arithmetic, Reading, and Standing

	PLASMA DENSITY (g/cm^3)		
	Mental Arithmetic (n=20)	Reading (n=20)	Standing (n=40)
Variable			
BLOOD DENSITY (g/cm^3)	.64 p <.001	.09	.69 p <.0001
TOTAL PLASMA PROTEIN (g/dl)	.44 p <.05	.21	.62 p <.001

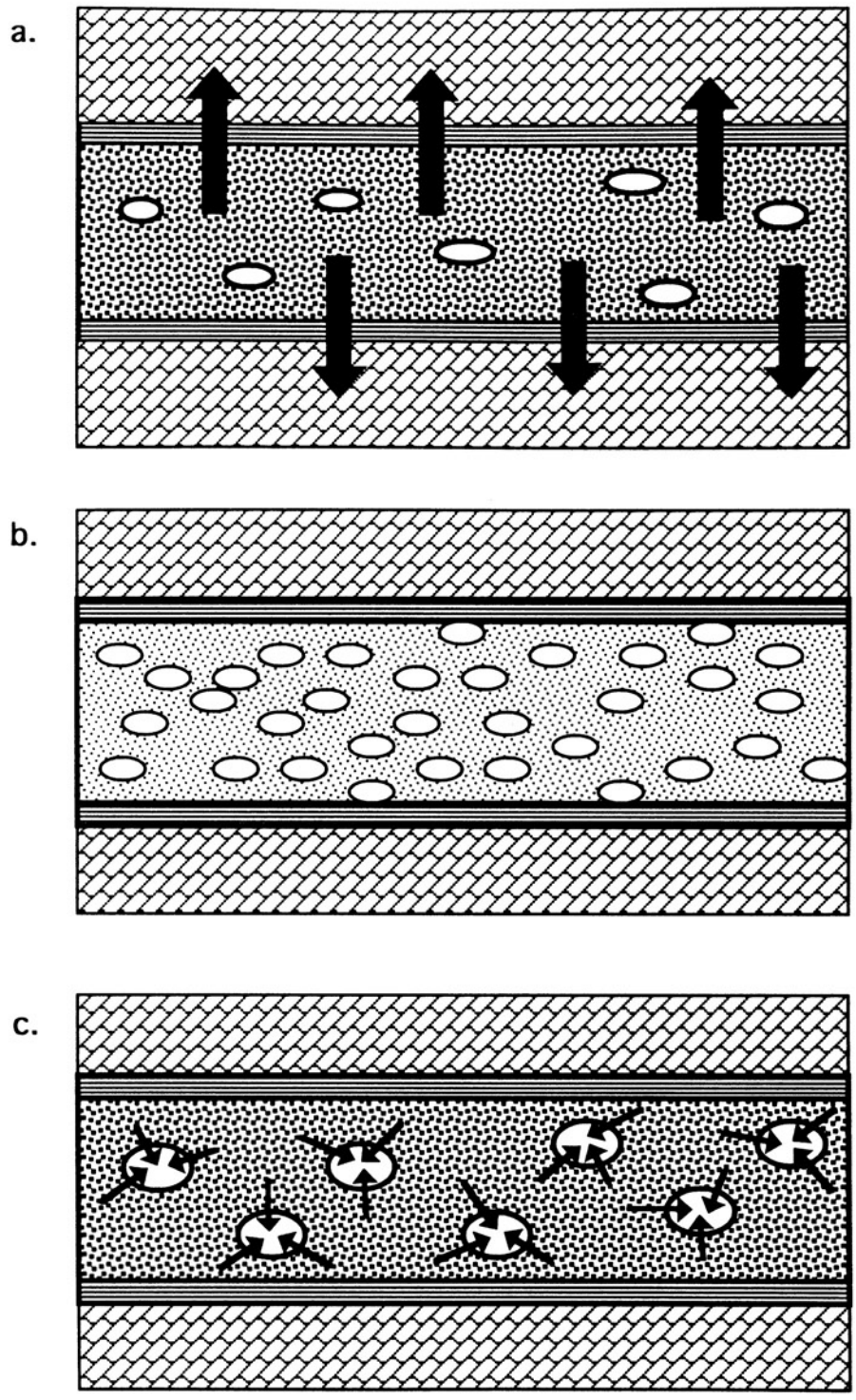


Figure 1. Diagrams of three potential mechanisms for stress-induced changes in plasma volume; transvascular fluid shifts (a), increased red cell mass (b), and transcellular fluid shifts (c).

Figure 2. Anxiety, anger, frustration, and irritation ratings during tasks in stress and no stress groups

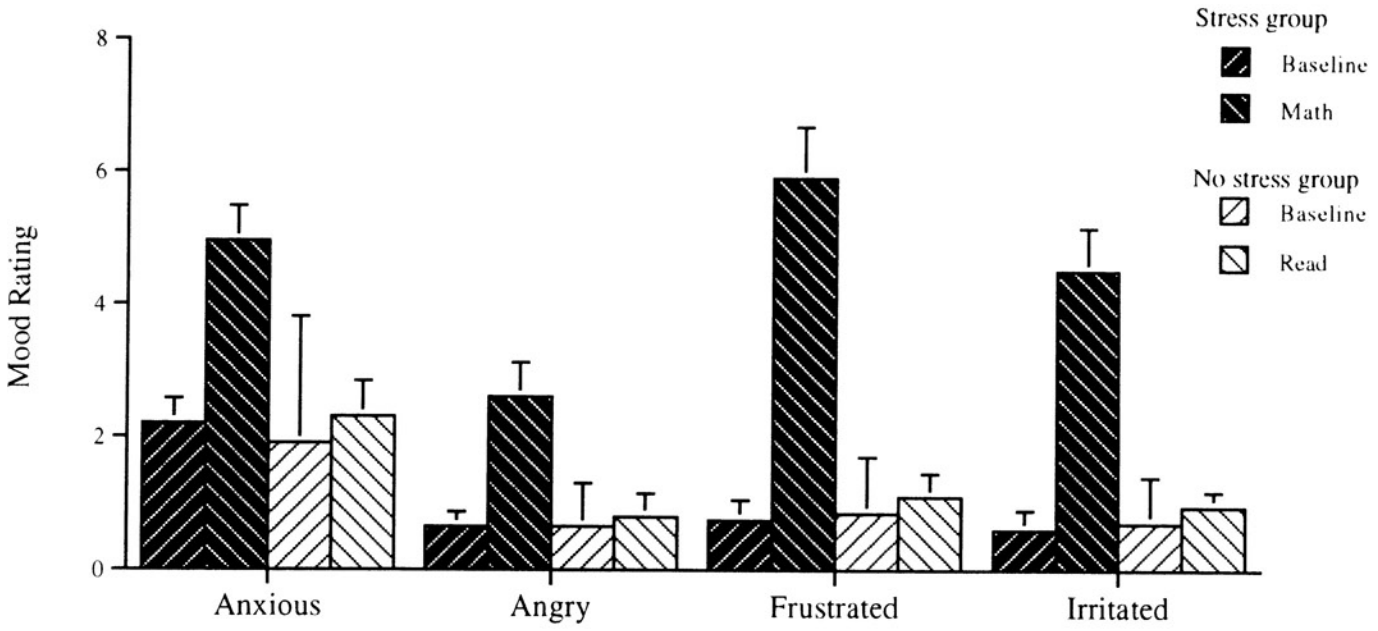


Figure 3. Difficult and challenging ratings during tasks in stress and no stress groups

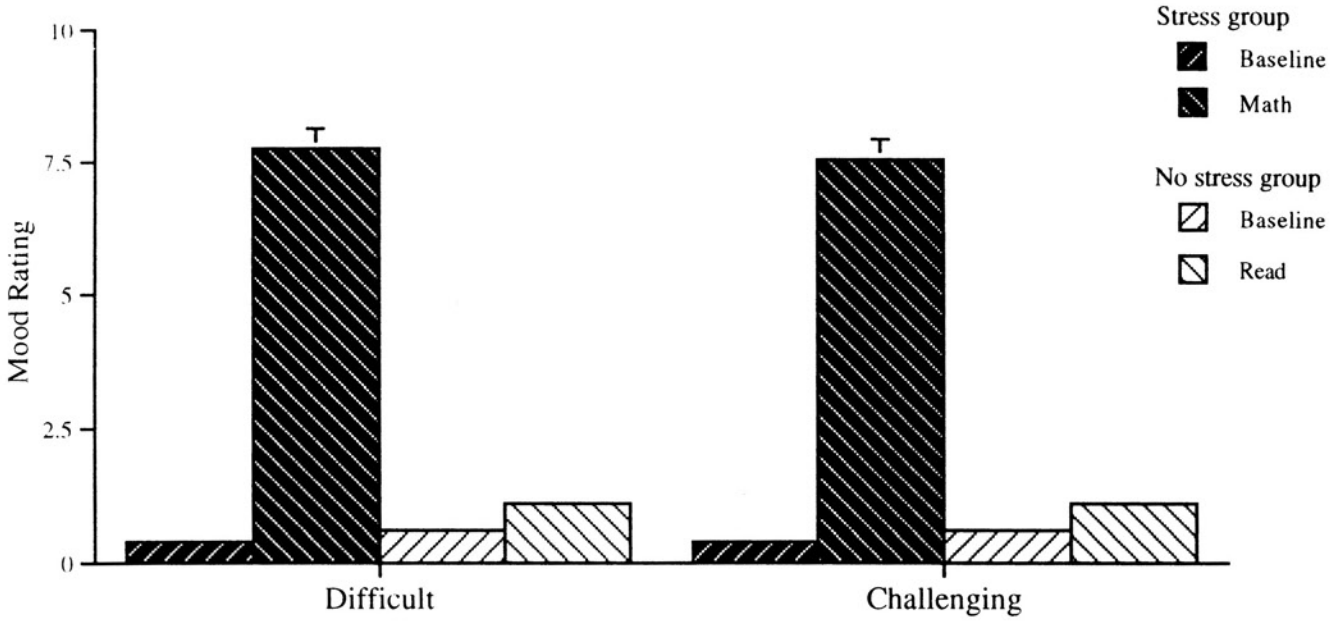


Figure 4. Boredom ratings for stress and no stress groups

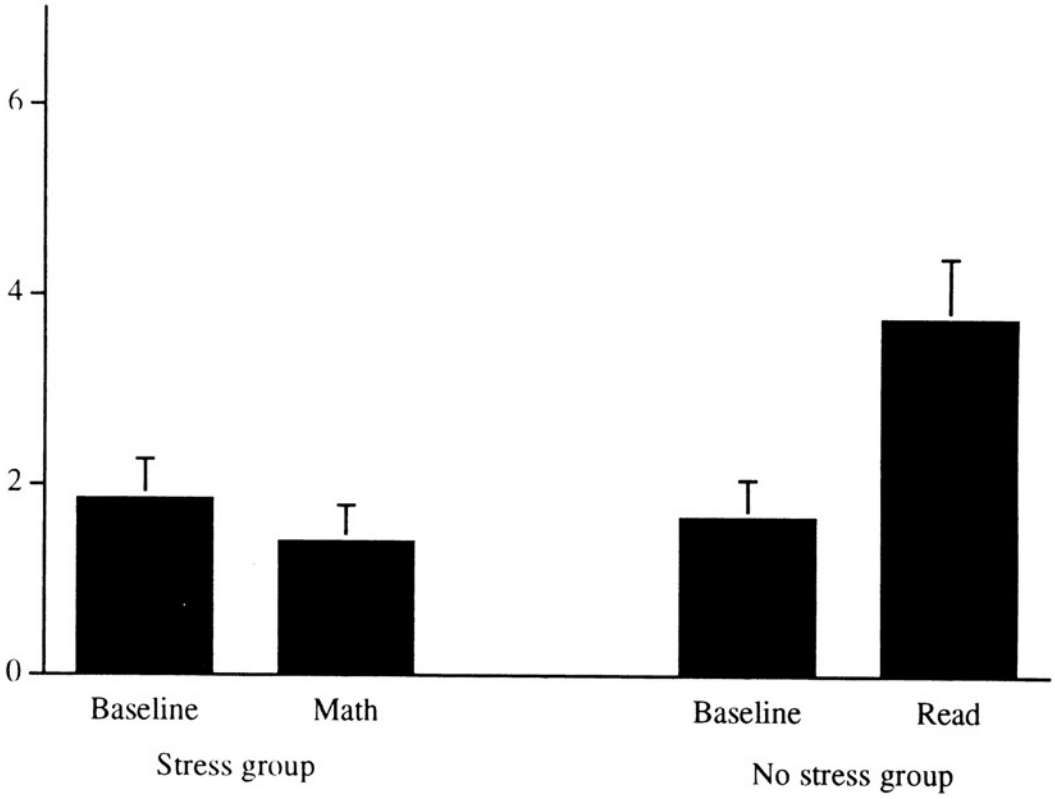


Figure 5. SBP during tasks in stress and no stress groups (mean±SE)

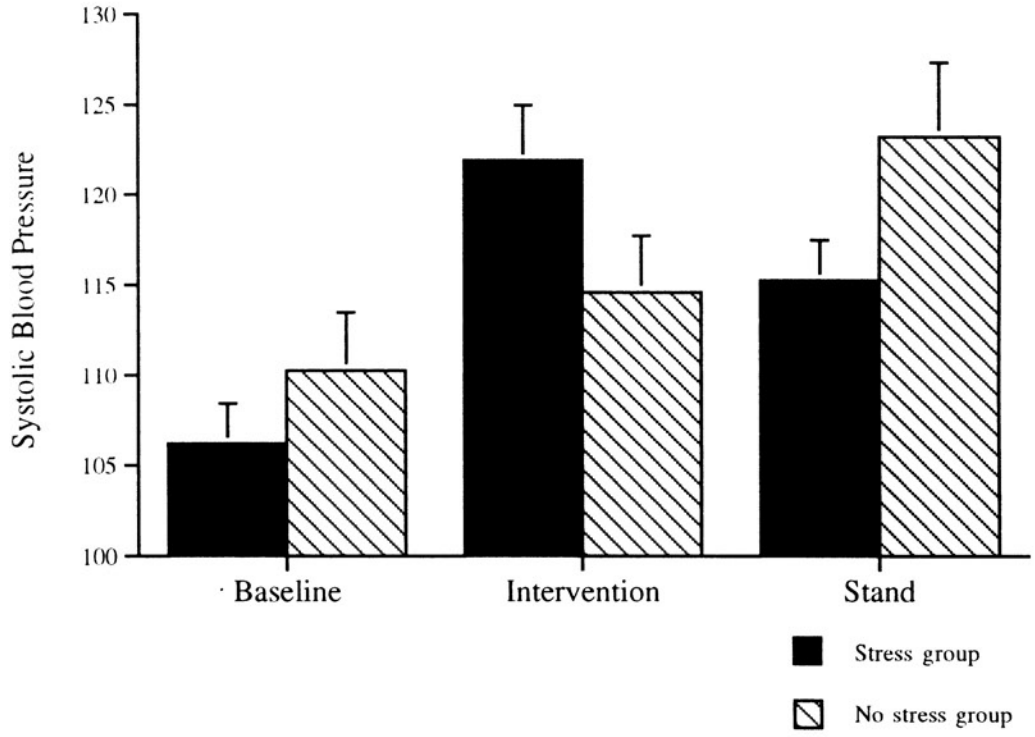


Figure 6. DBP during tasks in stress and no stress groups (mean±SE)

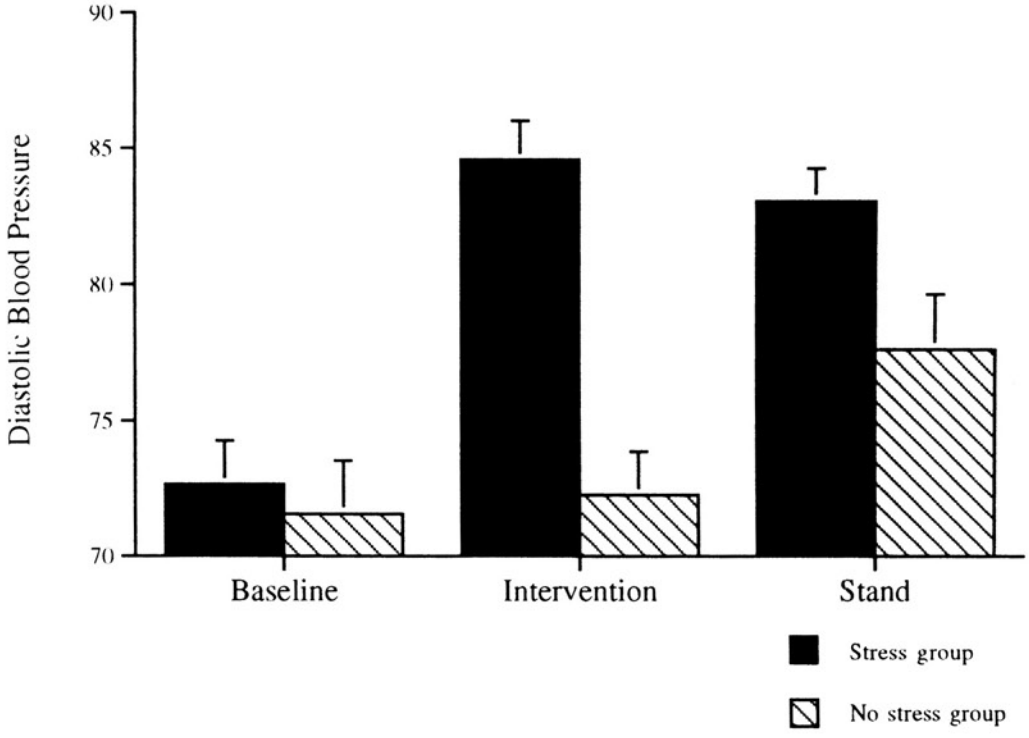


Figure 7. Mean arterial pressure during tasks in stress and no stress groups (mean±SE)

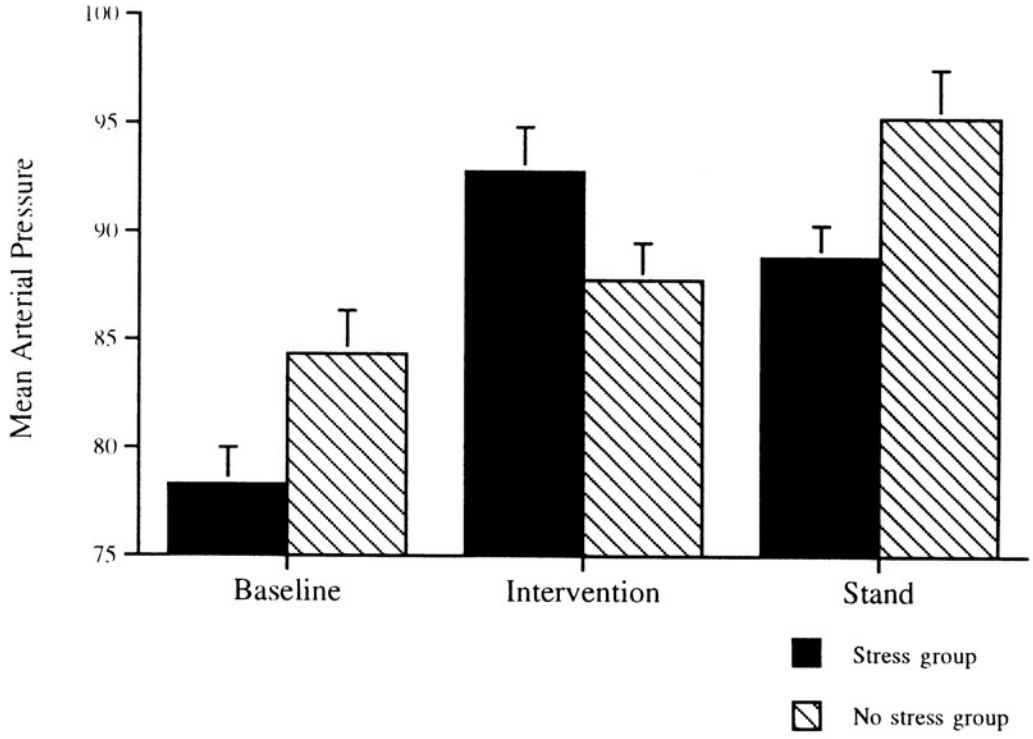


Figure 8. Plasma volume during tasks in stress and no stress groups (meam±SE)

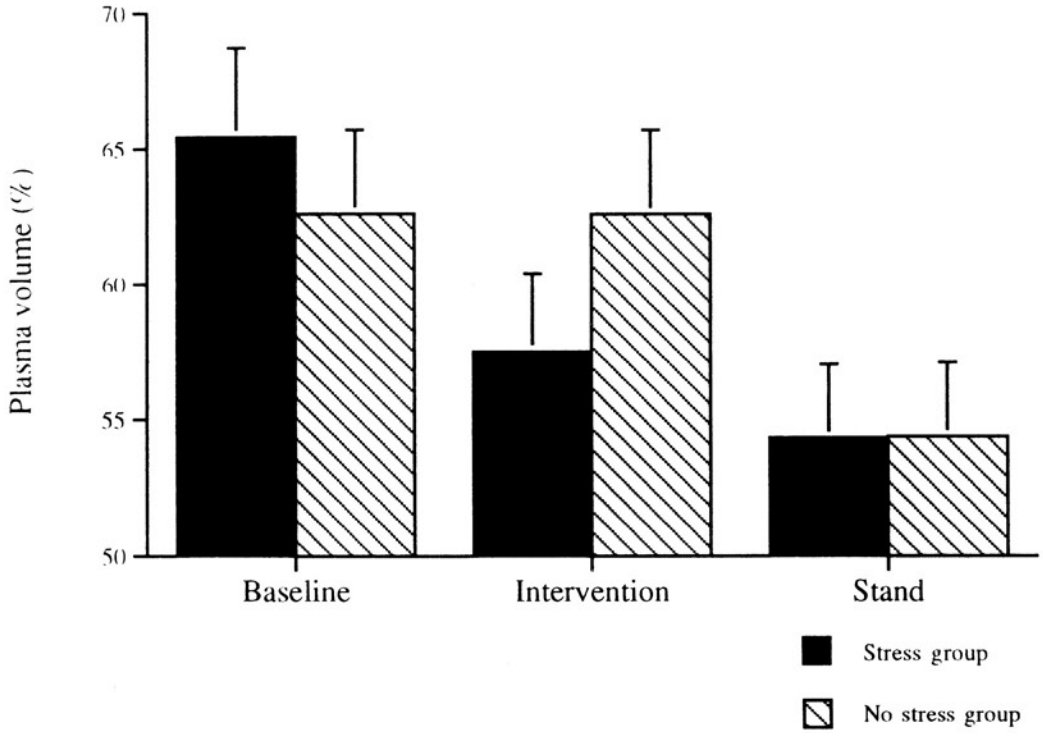


Figure 9. Blood density during tasks in stress and no stress groups (mean±SE)

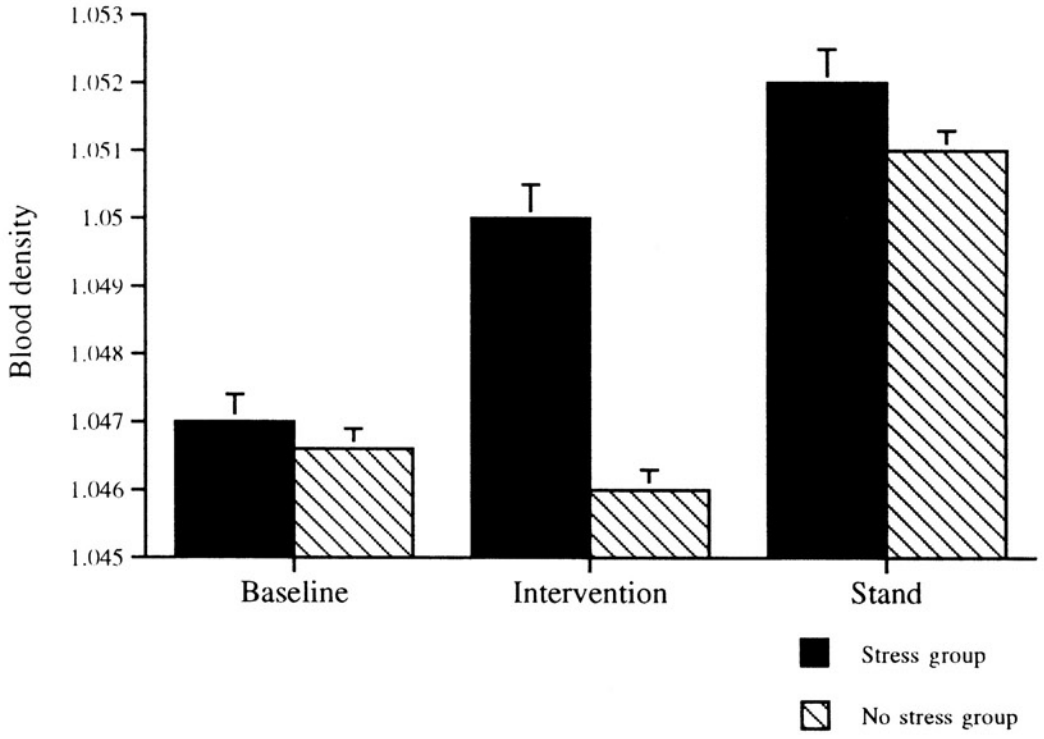


Figure 10. Plasma density during tasks in stress and no stress groups (mean±SE)

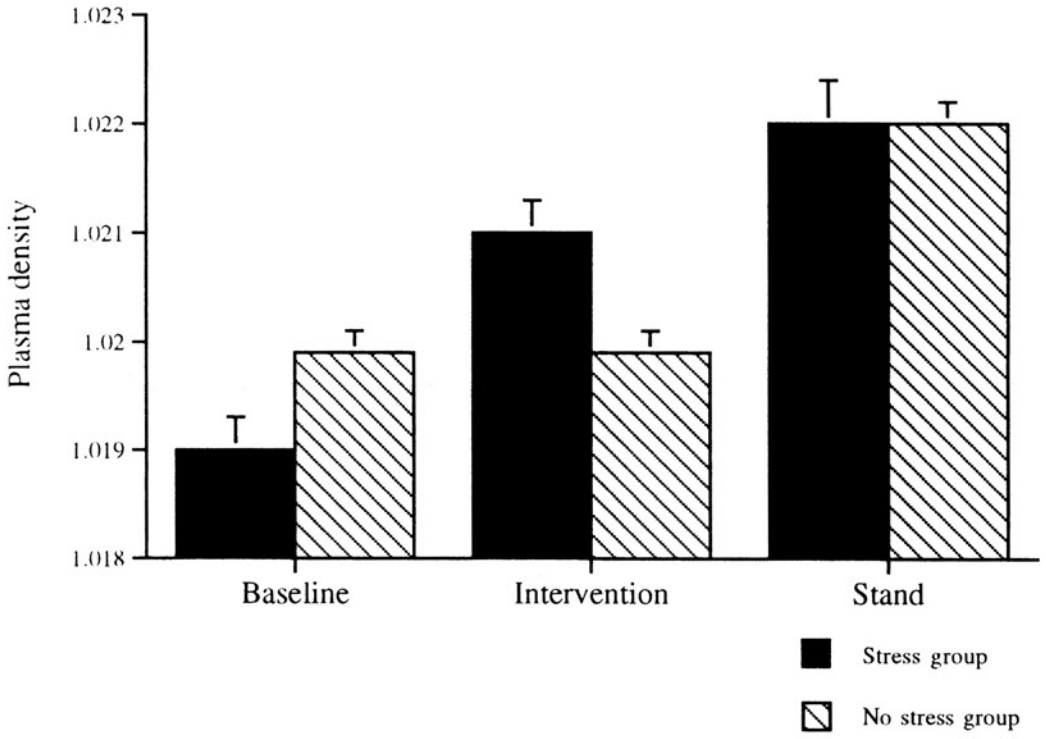


Figure 11. Total plasma protein during tasks in stress and no stress groups (mean±SE)

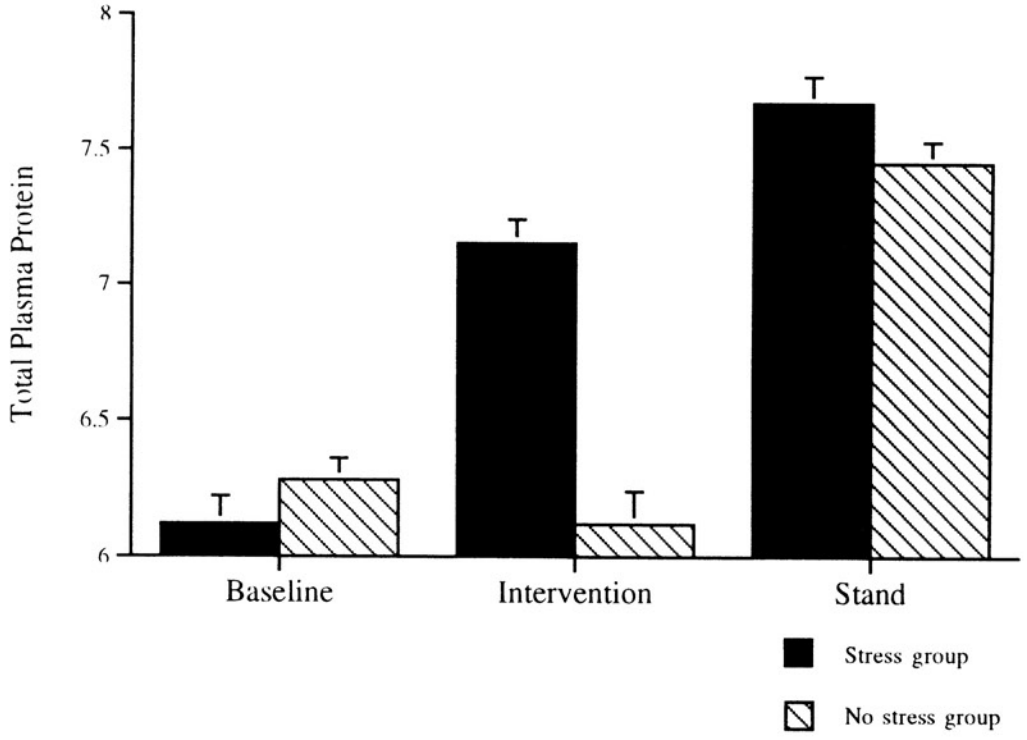


Figure 12. Mean corpuscular volume during tasks in stress and no stress groups (mean±SE)

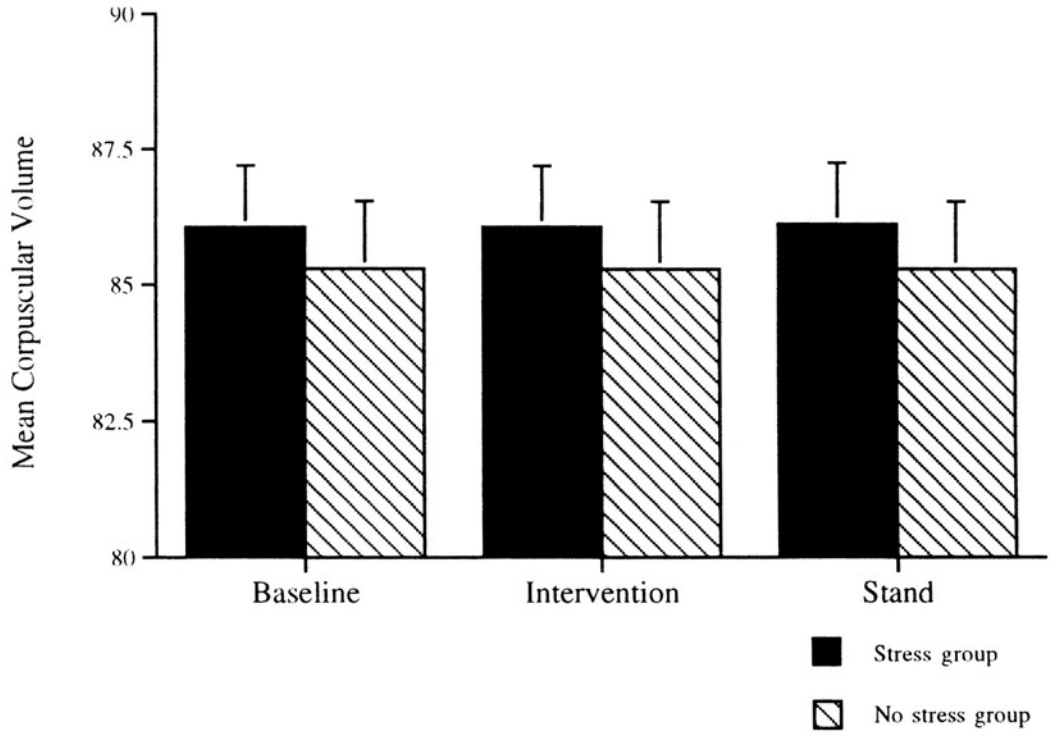
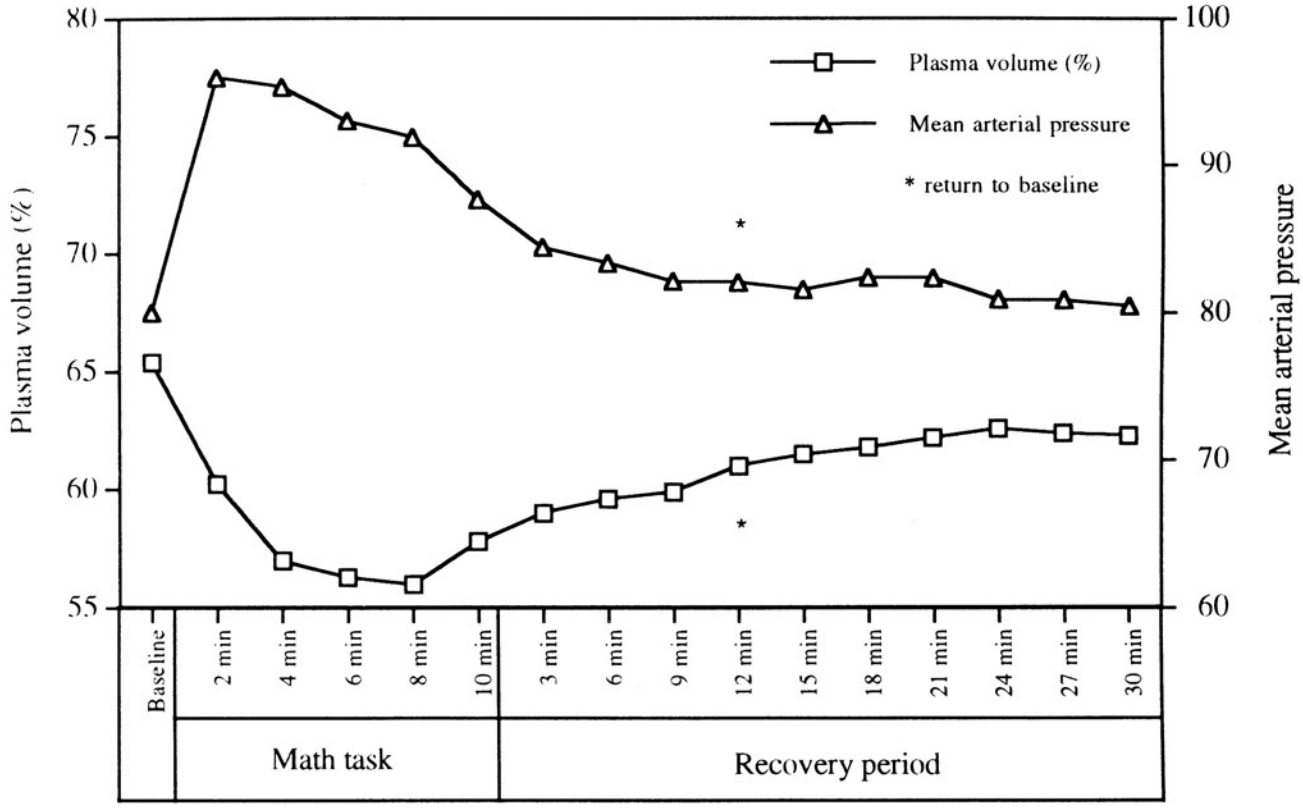


Figure 13. Time course of plasma volume and blood pressure changes during and after stress



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Appendix A

TELEPHONE SCREENING

DATE:_____

NAME:_____

ADDRESS:_____

PHONE: (Home)_____ (Work)_____

AGE:_____ Height:_____ Weight_____

OCCUPATION:_____

DO YOU HAVE ANY PERMANENT OR CHRONIC HEALTH PROBLEMS (eg. High BP, Asthma, Allergies, Heart Condition, Ulcer, Arthritis, Diabetes, Cancer...), OR ANY PROBLEMS WHICH HAVE LASTED FOR OVER 3 MONTHS: Y / N

IF YES, SPECIFY:_____

_____

DO YOU HAVE ANY PHYSICAL DISABILITIES THAT EFFECT GENERAL ACTIVITY LEVELS: Y / N

IF YES, SPECIFY:_____

HAS YOUR HEALTH CHANGED IN THE LAST 6 MONTHS: Y / N

IF YES, HOW:_____

DO YOU TAKE ANY PRESCRIPTION DRUGS: Y / N

IF YES, WHICH:_____

FOR WHAT HEALTH PROBLEMS:_____

DO YOU TAKE ANY NON-PRESCRIPTION DRUGS (ASPIRIN): Y / N

IF YES, WHICH:_____

FOR WHAT HEALTH PROBLEM:_____

DO YOU SMOKE: Y / N

IF YES, HOW MANY CIG./DAY:_____

DO YOU DRINK COFFEE OR CAFFEINATED SODA: Y / N

IF YES, HOW MUCH A DAY:_____

DO YOU DRINK BEER OR WINE: Y / N

IF YES, HOW MUCH A WEEK:_____

DO YOU DRINK ALCOHOLIC BEVERAGES OTHER THAN BEER OR WINE: Y/N

IF YES, HOW MUCH A WEEK:_____

DO YOU THINK YOU HAVE A DRINKING PROBLEM: Y / N

DO YOU OR HAVE YOU EVER TAKEN DRUGS SUCH AS MARIJUANA, COCAINE,
ETC: Y / N

ARE YOU PREGNANT: Y / N

ARE YOU CURRENTLY DIETING: Y / N

DO YOU EXERCISE REGULARLY: Y / N

IF YES, HOW MANY HOURS A WEEK:_____

ARE YOU CURRENTLY CONSULTING A PSYCHOLOGIST/PSYCHIATRIST: Y/N

IF YES, WHY:_____

If subject is eligible and interested in participating in the study, schedule for a session sometime in the a.m. Cannot be done on weekends. Give directions for coming to USUHS.

We will call them the day before the session to confirm the appointment and also to ask some questions about their current health status. Ask them where they can be reached that day.

Description of the study:

We are interested in the physiological responses to cognitive and physical tasks. We will be drawing blood (about two-thirds of a cup) and monitoring blood pressure throughout the study session. All subjects need to fast for 12 hours prior to their scheduled appointment and to abstain from drinking any caffeinated beverages on the morning of the appointment. Subjects need to drink 8 ounces of water within one hour of waking up on the morning of the study.

Appendix B

CONSENT FOR RESEARCH PARTICIPATION

Please read carefully

Title of Study: The effects of task performance on physiological functioning.
Addendum to protocol RO7265: Conditioned Reactivity in Vietnam Veterans.

We are studying the effects of task performance on several psychological and physiological functions including coping, immune function, heart rate, blood pressure, and hormone level changes. In order to do this we will have you answer a number of questions and participate in some tasks. We are asking you to help us by participating. You will be scheduled for one 3-hour session in our laboratory. We will pay you $25 for participating in this session.

We are interested in getting to know you and evaluating some of your attitudes, beliefs, and personal characteristics. In order to accomplish this, we will ask you a number of questions concerning your background. We will ask you questions about your health and well-being and administer some tasks measuring mental performance. We may ask you to complete any of the following simple tasks: playing a video game, listening to tapes of music, performing a proofreading task, watching films depicting surgery or disease, taking a simulated driving test, viewing scenes of unusual places, working on a color-word coordination task or a mental arithmetic task.

During the time you are in the laboratory we will be measuring your heart rate and blood pressure. In order to do this we will attach a cuff like the one used in your doctor's office to your dominant arm. This cuff is attached to a machine that will cause the cuff to inflate automatically at approximately 2-3 minute intervals at certain times throughout the session.

We will also need to draw samples of your blood. The blood will be drawn by a trained phlebotomist. A catheter, which is a needle with tubing, will be inserted into a forearm vein and will remain in place during the laboratory session. We will take about 2-3 teaspoons of blood each time we withdraw blood. We will collect blood twenty-three times during the session.

If you have donated blood within the past week, we will

not be able to draw blood from you. You will be in a sitting position when blood is drawn, and in the unlikely event of fainting,
smelling salts and proper positioning will be used for reviving. You may observe bruising at the site of the blood draw. The discoloration may last a few weeks. The blood will provide us with useful information about physiological changes in response to task performance.

Possible inconvenience or discomfort from this study involves possible frustration during the tasks. The blood drawing may be discomforting. There may be some minor bruising and possible dizziness, but the individuals who will be drawing blood are highly qualified and trained to minimize any discomfort and problems associated with the procedure. If at any time during the study you should choose not to participate in some part of the study, you may do so without penalty.

If you decide to participate, you may withdraw or discontinue participation at any time for any reason without prejudice. If you have any questions, we expect you to ask us.

Research records of your participation in this study will be maintained by the principal investigator. Confidentiality is protected to the best extent possible under law. Your identity will not be traceable by anyone other than the principal investigator. When you have completed the session and we have coded your data or you have withdrawn from the study, your name will be deleted from all records and no one will be able to trace your data. The data will be published in scientific journals but will not be published in any manner that can identify you.

This study does not entail any physical or mental risk beyond those described above. If, however you should become uncomfortable during the study, sufficiently uncomfortable that you would like to end the session, tell us. We do not expect this to occur, but if, for any reason, you feel that continuing would constitute a hardship, please tell us and we will end the session.

If you believe that you have suffered any injury or illness as a result of participating in this research, please contact Research Administration, 295-3303, at the University. This office can review the matter with you and may be able to identify resources available to you. Information about possible judicial avenues of compensation is available from the University's Legal Counsel, 295-3028.

If you desire additional information about this experiment, either about the rationale for it or its findings, or about your rights as a participant, you may call the

Department of Medical Psychology, 295-3270, to obtain information about it. In this way, you can make your participation in our research a more informative, educational experience. We welcome your comments and suggestions, and appreciate you willingness to help us.

YOU ARE MAKING A DECISION WHETHER OR NOT TO PARTICIPATE. YOUR SIGNATURE INDICATES THAT, HAVING READ THE ABOVE INFORMATION, YOU HAVE DECIDED TO PARTICIPATE.

_____
Date signed

_____
Subject initials

_____
Signature of Subject

_____
Social Security #

_____
Subject printed name/Status

I was present during the explanation referred to above, as well as during the volunteer's opportunity to ask questions. I hereby witness the Volunteer's signature.

_____
Witness Signature

_____
Printed Name/SSN

_____
Investigator or Designee signature

_____
Printed Name/SSN

Appendix C

Subject _____
Date _____

24-hour Food Intake Survey

1. What and when have you eaten in the last 24 hours?

_____

_____

2. Have you eaten within the last 12 hours? Yes____ No____

3. Did you drink 8 ounces of water within one hour after waking up this morning? Yes____ No____

Appendix D

1169	1251	1082	1188	2108	1337	1992
1162	1244	1065	1181	2091	1330	1985
1155	1237	1048	1174	2074	1323	1978
1148	1230	1031	1167	2057	1316	1971
1141	1223	1014	1160	2040	1309	1964
1134	1216	997	1153	2023	1302	1957
1127	1209	980	1146	2006	1295	1950
1120	1202	963	1139	1989	1288	1943
1113	1195	946	1132	1972	1281	1936
1106	1188	929	1125	1955	1274	1929
1099	1181	912	1118	1938	1267	1922
1092	1174	895	1111	1921	1260	1915
1085	1167	878	1104	1904	1253	1908
1078	1160	861	1097	1887	1246	1901
1071	1153	844	1090	1870	1239	1894
1064	1146	827	1083	1853	1232	1887
1057	1139	810	1076	1836	1225	1880
1050	1132	793	1069	1819	1218	1873
1043	1125	776	1062	1802	1211	1866
1036	1118	759	1055	1785	1204	1859
1029	1111	742	1048	1768	1197	1852
1022	1104	725	1041	1751	1190	1845
1015	1097	708	1034	1734	1183	1838
1008	1090	691	1027	1717	1176	1831
1001	1083	674	1020	1700	1169	1824

994	1076	657	1013	1683	1162	1817
987	1069	640	1006	1666	1155	1810
980	1062	623	999	1649	1148	1803
973	1055	606	992	1632	1141	1796
966	1048	689	985	1615	1134	1789
959	1041	672	978	1598	1127	1782
952	1034	655	971	1581	1120	1775
945	1027	638	964	1564	1113	1768
938	1020	611	957	1547	1106	1761
931	1013	594	950	1530	1099	1754
924	1006	577	943	1513	1092	1747
917	999	560	936	1496	1085	1740
910	992	543	929	1479	1078	1733
903	985	526	922	1462	1071	1726
896	978	509	915	1445	1064	1719
889	971	492	908	1428	1057	1712
882	964	475	901	1411	1050	1705
875	957	458	894	1394	1043	1698
868	950	431	887	1377	1036	1691
861	943	414	880	1360	1029	1684
854	936	397	873	1343	1022	1677
847	929	370	866	1326	1015	1670
840	922	353	859	1309	1008	1663
833	915	336	852	1292	1001	1656
826	908	319	845	1275	994	1649
819	901	302	838	1258	987	1642

Appendix E

Subject number _____
Date _____
Task _____

Instructions: Below are words which describe the feelings people have. Please read each one carefully and rate how much you have had that feeling during the past 10 minutes, including now. You may mark anywhere on each line.

	Not at all	Extremely
Happy	_____	
Bored	_____	
Anxious	_____	
Satisfied	_____	
Depressed	_____	
Interested	_____	
Angry	_____	
Frustrated	_____	
Restless	_____	
Irritated	_____	

How challenging did you find the task?

Not at all Extremely

_____

How difficult did you find the task?

Not at all Extremely

_____